PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 5/10, 5/16, 15/12, 15/62, 15/63, 15/64, C07K 14/705 — A1

(11) International Publication Number: WO 00/08133

(43) International Publication Date: 17 February 2000 (17.02.00)

(21) International Application Number: PCT/US99/17388

(22) International Filing Date: 2 August 1999 (02.08.99)

(30) Priority Data:
60/095,571 6 August 1998 (06.08.98) US

(71) Applicant *(for all designated States except US)*: MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: LIU, Qingyun [CN/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). McDONALD, Terrence, P. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). WANG, Ruiping [CN/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).

(74) Common Representative: MERCK & CO., INC.; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).

(81) Designated States: CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: NOVEL G PROTEIN–COUPLED RECEPTOR cDNA SEQUENCE

(57) Abstract

cDNA encoding a novel human G–protein coupled receptor, HG03, as well as the protein encoded by the cDNA, is provided. Methods of identifying agonists and antagonists of HG03 are also provided.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

TITLE OF THE INVENTION
NOVEL G PROTEIN-COUPLED RECEPTOR cDNA SEQUENCE

CROSS-REFERENCE TO RELATED APPLICATIONS

Not applicable.

STATEMENT REGARDING FEDERALLY-SPONSORED R&D

Not applicable.

REFERENCE TO MICROFICHE APPENDIX

Not applicable.

FIELD OF THE INVENTION

This invention relates to a novel human cDNA encoding a G protein-coupled receptor (GPCR) having homology to members of a family of receptors for nucleotides and platelet-activating factor, the protein encoded by the cDNA, and methods of identifying selective agonists and antagonists.

BACKGROUND OF THE INVENTION

G-protein coupled receptors (GPCRs) are a very large class of membrane receptors that relay information from the exterior to the interior of cells. GPCRs function by interacting with a class of heterotrimeric proteins known as G-proteins. Most GPCRs function by a similar mechanism. Upon the binding of agonist, a GPCR catalyzes the dissociation of guanosine diphosphate (GDP) from the α subunit of G proteins. This allows for the binding of guanosine triphosphate (GTP) to the α subunit, resulting in the disassociation of the α subunit from the β and γ subunits. The freed α subunit then interacts with other cellular components, and in the process passes on the extracellular signal represented by the presence of the agonist. Occasionally, it is the freed β and γ subunits which transduce the agonist signal.

GPCRs possess common structural characteristics. They have seven hydrophobic domains, about 20-30 amino acids long, linked by sequences of hydrophilic amino acids of varied length. These seven hydrophobic domains intercalate into the plasma membrane, giving rise to a protein with seven transmembrane domains, an extracellular amino terminus, and an intracellular

carboxy terminus (Strader et al., 1994, Ann. Rev. Biochem. 63:101-132; Schertler et al., 1993, Nature 362:770-7721; Dohlman et al., 1991, Ann. Rev. Biochem. 60:653-688).

GPCRs are expressed in a wide variety of tissue types and respond to a wide range of ligands, *e.g.*, protein hormones, biogenic amines, peptides, lipid derived messengers, etc. Given their wide range of expression and ligands, it is not surprising that GPCRs are involved in many pathological states. This has led to great interest in developing modulators of GPCR activity that can be used pharmacologically. For example, Table 1 of Stadel et al., 1997, Trends Pharmacol. Sci. 18:430-437, lists 37 different marketed drugs that act upon GPCRs. Accordingly, there is a great need to understand GPCR function and to develop agents that can be used to modulate GPCR activity.

SUMMARY OF THE INVENTION

The present invention is directed to a novel human cDNA that encodes a G-protein coupled receptor, HG03. The DNA encoding HG03 is substantially free from other nucleic acids and has the nucleotide sequence shown in SEQ.ID.NO.:1. Also provided is an HG03 protein encoded by the novel cDNA sequence. The HG03 protein is substantially free from other proteins and has the amino acid sequence shown in SEQ.ID.NO.:2. Methods of expressing HG03 in recombinant systems and of identifying agonists and antagonists of HG03 are provided.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the complete cDNA sequence of HG03 (SEQ.ID.NO.:1).

Figure 2 show the complete amino acid sequence of HG03 (SEQ.ID.:2).

Figure 3A-C shows the translation of HG03 open reading frame. The nucleotide sequence shown is (SEQ.ID.:1). The amino acid sequence shown is (SEQ.ID.:2).

Figure 4 shows the results of a Northern blot of the expression of HG03 mRNA in various human tissues.

Figure 5 shows the alignment of the amino acid sequence of HG03 with the amino acid sequence of the human platelet activating factor receptor (SEQ.ID.:3).

DETAILED DESCRIPTION OF THE INVENTION

For the purposes of this invention:

"Substantially free from other proteins" means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other proteins. Thus, an HG03 protein preparation that is substantially free from other proteins will contain, as a percent of its total protein, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non-HG03 proteins. Whether a given HG03 protein preparation is substantially free from other proteins can be determined by such conventional techniques of assessing protein purity as, *e.g.*, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) combined with appropriate detection methods, *e.g.*, silver staining or immunoblotting.

"Substantially free from other nucleic acids" means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other nucleic acids. Thus, an HG03 DNA preparation that is substantially free from other nucleic acids will contain, as a percent of its total nucleic acid, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non-HG03 nucleic acids. Whether a given HG03 DNA preparation is substantially free from other nucleic acids can be determined by such conventional techniques of assessing nucleic acid purity as, *e.g.*, agarose gel electrophoresis combined with appropriate staining methods, *e.g.*, ethidium bromide staining, or by sequencing.

"Functional equivalent" means a receptor which does not have the exact same amino acid sequence of a naturally occurring G protein-coupled receptor, due to alternative splicing, deletions, mutations, or additions, but retains at least 1%, preferably 10%, and more preferably 25% of the biological activity of the naturally occurring receptor. Such derivatives will have a significant homology with a natural GPCR and genes and cDNA encoding such derivatives can be detected by reduced stringency hybridization with a DNA sequence encoding a natural GPCR. The nucleic acid encoding a functional equivalent has at least about 50% homology at the nucleotide level to a naturally occurring receptor nucleic acid.

A polypeptide has "substantially the same biological activity" as HG03 if that polypeptide has a K_d for a ligand that is no more than 5-fold greater than the K_d of HG03 for the same ligand.

A "conservative amino acid substitution" refers to the replacement of one amino acid residue by another, chemically similar, amino acid residue. Examples of such conservative substitutions are: substitution of one hydrophobic residue (isoleucine, leucine, valine, or methionine) for another; substitution of one polar residue for another polar residue of the same charge (*e.g.*, arginine for lysine; glutamic acid for aspartic acid).

One aspect of this invention is the identification and cloning of a novel G protein-coupled receptor (GPCR), substantially free from receptor associated proteins, designated as HG03.

Another aspect of this invention is nucleic acids which encode the HG03 G protein-coupled receptor. These nucleic acids are substantially free from associated nucleic acids. For most cloning purposes, DNA is a preferred nucleic acid.

The present invention provides a cDNA molecule substantially free from other nucleic acids having the nucleotide sequence shown in FIGURE 1 as SEQ.ID.NO.:1. Analysis of FIGURE 3A-C revealed that SEQ.ID.NO:1 contains an open reading frame at positions 346-1419. Thus, the present invention also provides a cDNA molecule substantially free from other nucleic acids comprising the nucleotide sequence of positions 346-1419 of SEQ.ID.NO.:1. The present invention also provides recombinant DNA molecules comprising the nucleotide sequence of positions 346-1419 of SEQ.ID.NO.:1.

Sequence analysis of the open reading frame of the HG03 cDNA revealed that it encodes a protein of 358 amino acids. Based on its predicted amino acid sequence, HG03 most likely represents a novel GPCR. Northern blot analysis showed that HG03 RNA is highly expressed in the prostate, placenta, and trachea in human with a major transcript of ~1.8 kb and a minor transcript of ~8.0 kb. HG03 was also expressed at lower levels in thymus and testis as a transcript of ~1.8 kb. HG03 appears to be related to the members of receptors for nucleotides and platelet-activating factor.

The novel DNA sequences of the present invention encoding HG03, in whole or in part, can be linked with other DNA sequences, *i.e.*, DNA sequences to which HG03 is not naturally linked, to form "recombinant DNA molecules" containing HG03. The novel DNA sequences of the present invention can be inserted into vectors which comprise nucleic acids encoding a GPCR or a functional equivalent. These vectors may be comprised of DNA or RNA; for most cloning purposes DNA vectors are preferred. Typical vectors include plasmids, modified viruses, bacteriophage and cosmids, yeast artificial chromosomes and other forms of episomal or integrated DNA that can encode a GPCR. It is well within the skilled artisan to determine an appropriate vector for a particular gene transfer or other use.

Included in the present invention are cDNA sequences that hybridize to SEQ.ID.NO.:1 under stringent conditions. By way of example, and not limitation, a procedure using conditions of high stringency is as follows: Prehybridization of filters containing DNA is carried out for 2 hr. to overnight at 65°C in buffer composed of 6X SSC, 5X Denhardt's solution, and 100 µg/ml denatured salmon sperm DNA. Filters are hybridized for 12 to 48 hrs at 65°C in prehybridization mixture containing 100 µg/ml denatured salmon sperm DNA and 5-20 X 10^6 cpm of ^{32}P-labeled probe. Washing of filters is done at 37°C for 1 hr in a solution containing 2X SSC, 0.1% SDS. This is followed by a wash in 0.1X SSC, 0.1% SDS at 50°C for 45 min. before autoradiography.

Other procedures using conditions of high stringency would include either a hybridization step carried out in 5XSSC, 5X Denhardt's solution, 50% formamide at 42°C for 12 to 48 hours or a washing step carried out in 0.2X SSPE, 0.2% SDS at 65°C for 30 to 60 minutes.

Reagents mentioned in the foregoing procedures for carrying out high stringency hybridization are well known in the art. Details of the composition of these reagents can be found in, *e.g.*, Sambrook, Fritsch, and Maniatis, 1989, Molecular Cloning: A Laboratory Manual, second edition, Cold Spring Harbor Laboratory Press. In addition to the foregoing, other conditions of high stringency which may be used are well known in the art.

Another aspect of the present invention includes host cells that have been engineered to contain and/or express DNA sequences encoding HG03. Such

recombinant host cells can be cultured under suitable conditions to produce HG03. An expression vector containing DNA encoding HG03 can be used for expression of HG03 in a recombinant host cell. Recombinant host cells may be prokaryotic or eukaryotic, including but not limited to, bacteria such as *E. coli*, fungal cells such as yeast, mammalian cells including, but not limited to, cell lines of human, bovine, porcine, monkey and rodent origin, and insect cells including but not limited to *Drosophila* and silkworm derived cell lines. Cell lines derived from mammalian species which are suitable for recombinant expression of HG03 and which are commercially available, include but are not limited to, L cells L-M(TK$^-$) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) and MRC-5 (ATCC CCL 171).

Human embryonic kidney (HEK 293) cells and Chinese hamster ovary (CHO) cells are particularly suitable for expression of the HG03 protein because these cells express a large number of G-proteins. Thus, it is likely that at least one of these G-proteins will be able to functionally couple the signal generated by interaction of HG03 and its ligands, thus transmitting this signal to downstream effectors, eventually resulting in a measurable change in some assayable component, *e.g.*, cAMP level, expression of a reporter gene, hydrolysis of inositol lipids, or intracellular Ca^{2+} levels.

A variety of mammalian expression vectors can be used to express recombinant HG03 in mammalian cells. Commercially available mammalian expression vectors which are suitable include, but are not limited to, pCR2.1 (Invitrogen), pMC1neo (Stratagene), pSG5 (Stratagene), pcDNAI and pcDNAIamp, pcDNA3, pcDNA3.1, pCR3.1 (Invitrogen), EBO-pSV2-neo (ATCC 37593), pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), and pSV2-dhfr (ATCC 37146). Following expression in recombinant cells, HG03 can be purified by conventional techniques to a level that is substantially free from other proteins.

As with many receptor proteins, it is possible to modify many of the amino acids of HG03, particularly those which are not found in the ligand binding domain, and still retain substantially the same biological activity as the original receptor. Thus this invention includes modified HG03 polypeptides which have

amino acid deletions, additions, or substitutions but that still retain substantially the same biological activity as HG03. It is generally accepted that single amino acid substitutions do not usually alter the biological activity of a protein (see, *e.g.*, Molecular Biology of the Gene, Watson *et al.*, 1987, Fourth Ed., The Benjamin/Cummings Publishing Co., Inc., page 226; and Cunningham & Wells, 1989, Science 244:1081-1085). Accordingly, the present invention includes polypeptides where one amino acid substitution has been made in SEQ.ID.NO.:2 wherein the polypeptides still retain substantially the same biological activity as HG03. The present invention also includes polypeptides where two or more amino acid substitutions have been made in SEQ.ID.NO.:2 wherein the polypeptides still retain substantially the same biological activity as HG03. In particular, the present invention includes embodiments where the above-described substitutions are conservative substitutions. In particular, the present invention includes embodiments where the above-described substitutions do not occur in the ligand-binding domain of HG03.

The present invention also includes C-terminal truncated forms of HG03, particularly those which encompass the extracellular portion of the receptor, but lack the intracellular signaling portion of the receptor. Such truncated receptors are useful in various binding assays described herein, for crystallization studies, and for structure-activity-relationship studies.

Romano *et al.*, 1996, J. Biol. Chem. 271:28612-28616 demonstrated that some GPCRs are often found as homodimers formed by an intermolecular disulfide bond. The location of the cysteines responsible for the disulfide bond was found to be in the amino terminal 17 kd of the receptors. Accordingly, the present invention includes dimers of HG03 proteins.

It has been found that, in some case, membrane spanning regions of receptor proteins can be used to inhibit receptor function (Ng *et al.*, 1996, Biochem. Biophys. Res. Comm. 227:200-204; Hebert *et al.*, 1996, J. Biol. Chem. 271, 16384-16392; Lofts *et al.*, Oncogene 8:2813-2820). Accordingly, the present invention provides peptides derived from the seven membrane spanning regions of HG03 and their use to inhibit HG03 function. Such peptides can include the whole or parts of the receptor membrane spanning domains.

The present invention also includes chimeric HG03 proteins. Chimeric HG03 proteins consist of a contiguous polypeptide sequence of HG03 fused in frame

to a polypeptide sequence of a non-HG03 protein. For example, the N-terminal domain and seven transmembrane spanning domains of HG03 fused at the C-terminus in frame to a G protein would be a chimeric HG03 protein.

The present invention also includes HG03 proteins that are in the form of multimeric structures, *e.g.*, dimers. Such multimers of other G-protein coupled receptors are known (Hebert *et al.*, 1996, J. Biol. Chem. 271, 16384-16392; Ng *et al.*, 1996, Biochem. Biophys. Res. Comm. 227, 200-204; Romano *et al.*, 1996, J. Biol. Chem. 271, 28612-28616).

The present invention also includes isolated forms of HG03 proteins. By "isolated HG03 protein" is meant HG03 protein that has been isolated from a natural source. Use of the term "isolated" indicates that HG03 protein has been removed from its normal cellular environment. Thus, an isolated HG03 protein may be in a cell-free solution or placed in a different cellular environment from that in which it occurs naturally. The term isolated does not imply that an isolated HG03 protein is the only protein present, but instead means that an isolated HG03 protein is at least 95% free of non-amino acid material (*e.g.*, nucleic acids, lipids, carbohydrates) naturally associated with the HG03 protein. Thus, an HG03 protein that is expressed in bacteria or even in eukaryotic cells which do not naturally (*i.e.*, without human intervention) express it through recombinant means is an "isolated HG03 protein."

The specificity of binding of compounds showing affinity for HG03 is shown by measuring the affinity of the compounds for recombinant cells expressing the cloned receptor or for membranes from these cells. Expression of the cloned receptor and screening for compounds that bind to HG03 or that inhibit the binding of a known, radiolabeled ligand of HG03 to these cells, or membranes prepared from these cells, provides an effective method for the rapid selection of compounds with high affinity for HG03. Such ligands need not necessarily be radiolabeled but can also be nonisotopic compounds that can be used to displace bound radiolabeled compounds or that can be used as activators in functional assays. Compounds identified by the above method are likely to be agonists or antagonists of HG03 and may be peptides, proteins, or non-proteinaceous organic molecules.

Therefore, the present invention include assays by which HG03 agonists and antagonists may be identified. Methods for identifying agonists and antagonists of other receptors are well known in the art and can be adapted to identify agonists and antagonists of HG03. For

example, Cascieri *et al.*, 1992, Molec. Pharmacol. 41:1096-1099 describe a method for identifying substances that inhibit agonist binding to rat neurokinin receptors and thus are potential agonists or antagonists of neurokinin receptors. The method involves transfecting COS cells with expression vectors containing rat neurokinin receptors, allowing the transfected cells to grow for a time sufficient to allow the neurokinin receptors to be expressed, harvesting the transfected cells and resuspending the cells in assay buffer containing a known radioactively labeled agonist of the neurokinin receptors either in the presence or the absence of the substance, and then measuring the binding of the radioactively labeled known agonist of the neurokinin receptor to the neurokinin receptor. If the amount of binding of the known agonist is less in the presence of the substance than in the absence of the substance, then the substance is a potential agonist or antagonist of the neurokinin receptor.

Accordingly, the present invention includes a method for determining whether a substance is a potential agonist or antagonist of HG03 that comprises:

(a) transfecting cells with an expression vector encoding HG03;

(b) allowing the transfected cells to grow for a time sufficient to allow HG03 to be expressed;

(c) harvesting the transfected cells and resuspending the cells in the presence of a known labeled agonist of HG03 in the presence and in the absence of the substance;

(d) measuring the binding of the labeled agonist to HG03;

where if the amount of binding of the known agonist is less in the presence of the substance than in the absence of the substance, then the substance is a potential agonist or antagonist of HG03.

In a modification of the above-described method, step (b) is modified in that the cells are stably transfected with the expression vector containing HG03. In another modification of the above-described method, step (c) is modified in that the cells are not harvested and resuspended but rather the radioactively labeled known agonist and the substance are

contacted with the cells while the cells are attached to a substratum, *e.g.*, tissue culture plates.

The conditions under which step (c) of the method is practiced are conditions that are typically used in the art for the study of protein-ligand interactions: *e.g.*, physiological pH; salt conditions such as those represented by such commonly used buffers as PBS or in tissue culture media; a temperature of about 4°C to about 55°C.

In a particular embodiment of the above-described method, the cells are eukaryotic cells. In another embodiment, the cells are mammalian cells. In other embodiments, the cells are L cells L-M(TK-) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) or MRC-5 (ATCC CCL 171).

The present invention also includes a method for determining whether a substance is capable of binding to HG03, *i.e.*, whether the substance is a potential agonist or an antagonist of HG03, where the method comprises:

(a) providing test cells by transfecting cells with an expression vector that directs the expression of HG03 in the cells;

(b) exposing the test cells to the substance;

(c) measuring the amount of binding of the substance to HG03;

(d) comparing the amount of binding of the substance to HG03 in the test cells with the amount of binding of the substance to control cells that have not been transfected with HG03;

wherein if the amount of binding of the substance is greater in the test cells as compared to the control cells, the substance is capable of binding to HG03. Determining whether the substance is actually an agonist or antagonist can then be accomplished by the use of functional assays such as, *e.g.*, the assay involving the use of promiscuous G-proteins described below.

The conditions under which step (b) of the method is practiced are conditions that are typically used in the art for the study of protein-ligand interactions: *e.g.*, physiological pH; salt conditions such as those represented by such commonly used buffers as PBS or in tissue culture media; a temperature of about 4°C to about 55°C.

The assays described above can be carried out with cells that have been transiently or stably transfected with HG03. Transfection is meant to include any method known in the art for introducing HG03 into the test cells. For example, transfection includes calcium phosphate or calcium chloride mediated transfection, lipofection, infection with a retroviral construct containing HG03, and electroporation.

Where binding of the substance or agonist to HG03 is measured, such binding can be measured by employing a labeled substance or agonist. The substance or agonist can be labeled in any convenient manner known to the art, *e.g.*, radioactively, fluorescently, enzymatically.

In particular embodiments of the above-described methods, HG03 has an amino acid sequence of SEQ.ID.NO.:2.

The above-described methods can be modified in that, rather than exposing the test cells to the substance, membranes can be prepared from the test cells and those membranes can be exposed to the substance. Such a modification utilizing membranes rather than cells is well known in the art and is described in, *e.g.*, Hess *et al.*, 1992, Biochem. Biophys. Res. Comm. 184:260-268.

Accordingly, the present invention provides a method for determining whether a substance is capable of binding to HG03 comprising:

(a) providing test cells by transfecting cells with an expression vector that directs the expression of HG03 in the cells;

(b) preparing membranes containing HG03 from the test cells and exposing the membranes from the test cells to the substance;

(c) measuring the amount of binding of the substance to the HG03 in the membranes from the test cells;

(d) comparing the amount of binding of the substance to HG03 in the membranes from the test cells with the amount of binding of the substance to membranes from control cells that have not been transfected with HG03;

where HG03 has an amino acid sequence of SEQ.ID.NO.:2;

where if the amount of binding of the substance to HG03 in the membranes from the test cells is greater than the amount of binding of the substance to the membranes from the control cells, then the substance is capable of binding to HG03.

The present invention provides a method for determining whether a substance is capable of binding to HG03 comprising:

(a) providing test cells by transfecting cells with an expression vector that directs the expression of HG03 in the cells;

(b) exposing the test cells to a ligand of HG03 under conditions such that the ligand binds to the HG03 in the test cells;

(c) subsequently or concurrently to step (b), exposing the cells to a substance that is suspected of being capable of binding to HG03;

(d) measuring the amount of binding of the ligand to HG03 in the presence and the absence of the substance;

(e) comparing the amount of binding of the ligand to HG03 in the presence and the absence of the substance where a decrease in the amount of binding of the ligand to HG03 in the presence of the substance indicates that the substance is capable of binding to HG03;

where HG03 has an amino acid sequence of SEQ.ID.NO.:2.

The present invention provides a method for determining whether a substance is capable of binding to HG03 comprising:

(a) providing test cells by transfecting cells with an expression vector that directs the expression of HG03 in the cells;

(b) preparing membranes containing HG03 from the test cells and exposing the membranes to a ligand of HG03 under conditions such that the ligand binds to the HG03 in the membranes;

(c) subsequently or concurrently to step (b), exposing the membranes from the test cells to a substance;

(d) measuring the amount of binding of the ligand to the HG03 in the membranes in the presence and the absence of the substance;

(e) comparing the amount of binding of the ligand to HG03 in the membranes in the presence and the absence of the substance where a decrease in the amount of binding of the ligand to HG03 in the membranes in the presence of the substance indicates that the substance is capable of binding to HG03;

where HG03 has an amino acid sequence of SEQ ID NO.:2.

As a further modification of the above-described methods, RNA encoding HG03 can be prepared as, *e.g.*, by *in vitro* transcription using a plasmid containing HG03 under the control of a bacteriophage T7 promoter, and the RNA can be microinjected into *Xenopus* oocytes in order to cause the expression of HG03 in the oocytes. Substances are then tested for binding to the HG03 expressed in the oocytes. Alternatively, rather than detecting binding, the effect of the substances on the electrophysiological properties of the oocytes can be determined.

The present invention includes assays by which HG03 agonists and antagonists may be identified by their ability to stimulate or antagonize a functional response mediated by HG03. HG03 belongs to the class of proteins known as G-protein coupled receptors (GPCRs). GPCRs transmit signals across cell membranes upon the binding of ligand. The ligand-bound GPCR interacts with a heterotrimeric G-protein, causing the Gα subunit of the G-protein to disassociate from the Gβ and Gγ subunits. The Gα subunit can then go on to activate a variety of second messenger systems.

Generally, a particular GPCR is only coupled to a particular type of G-protein. Thus, to observe a functional response from the GPCR, it is necessary to ensure that the proper G-protein is present in the system containing the GPCR. It has been found, however, that there are certain G-proteins that are "promiscuous." These promiscuous G-proteins will couple to, and thus transduce a functional signal from, virtually any GPCR. See Offermanns & Simon, 1995, J. Biol. Chem. 270:15175, 15180 (Offermanns). Offermanns described a system in which cells are transfected with expression vectors that result in the expression of one of a large number of GPCRs as well as the expression of one of the promiscuous G-proteins Gα15 or Gα16. Upon the addition of an agonist of the GPCR to the transfected cells, the GPCR was activated and was able,

via Gα15 or Gα16, to activate the β isoform of phospholipase C, leading to an increase in inositol phosphate levels in the cells.

Therefore, by making use of these promiscuous G-proteins as in Offermanns, it is possible to set up functional assays for HG03, even in the absence of knowledge of the G-protein with which HG03 is coupled *in vivo*. One possibility is to create a fusion or chimeric protein composed of the extracellular and membrane spanning portion of HG03 fused to a promiscuous G-protein. Such a fusion protein would be expected to transduce a signal following binding of ligand to the HG03 portion of the fusion protein. Accordingly, the present invention provides a method of identifying antagonists of HG03 comprising:

(a) providing cells that expresses a chimeric HG03 protein fused at its C-terminus to a promiscuous G-protein;

(b) exposing the cells to an agonist of HG03;

(c) subsequently or concurrently to step (b), exposing the cells to a substance that is a suspected antagonist of HG03;

(d) measuring the level of inositol phosphates in the cells;

where a decrease in the level of inositol phosphates in the cells in the presence of the substance as compared to the level of inositol phosphates in the cells in the absence of the substance indicates that the substance is an antagonist of HG03.

Another possibility for utilizing promiscuous G-proteins in connection with HG03 includes a method of identifying agonists of HG03 comprising:

(a) providing cells that expresses both HG03 and a promiscuous G-protein;

(b) exposing the cells to a substance that is a suspected agonist of HG03;

(c) measuring the level of inositol phosphates in the cells;

where an increase in the level of inositol phosphates in the cells as compared to the level of inositol phosphates in the cells in the absence of the suspected agonist indicates that the substance is an agonist of HG03.

Levels of inositol phosphates can be measured by monitoring calcium mobilization. Intracellular calcium mobilization is typically

assayed in whole cells under a microscope using fluorescent dyes or in cell suspensions via luminescence using the aequorin assay.

In a particular embodiment of the above-described method, the cells are eukaryotic cells. In another embodiment, the cells are mammalian cells. In other embodiments, the cells are L cells L-M(TK$^-$) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616). BS-C-1 (ATCC CCL 26) or MRC-5 (ATCC CCL 171).

In a particular embodiment of the above-described method, the cells are transfected with expression vectors that direct the expression of HG03 and the promiscuous G-protein in the cells.

The conditions under which step (b) of the method is practiced are conditions that are typically used in the art for the study of protein-ligand interactions: *e.g.*, physiological pH; salt conditions such as those represented by such commonly used buffers as PBS or in tissue culture media; a temperature of about 4°C to about 55°C.

In a particular embodiment of the above-described method, the promiscuous G-protein is selected from the group consisting of Gα15 or Gα16. Expression vectors containing Gα15 or Gα16 are known in the art. See, *e.g.*, Offermanns; Buhl *et al.*, 1993, FEBS Lett. 323:132-134; Amatruda *et al.*, 1993, J. Biol. Chem. 268:10139-10144.

The above-described assay can be easily modified to form a method to identify antagonists of HG03. Such a method is also part of the present invention and comprises:

(a) providing cells that expresses both HG03 and a promiscuous G-protein;

(b) exposing the cells to a substance that is an agonist of HG03;

(c) subsequently or concurrently to step (b), exposing the cells to a substance that is a suspected antagonist of HG03;

(d) measuring the level of inositol phosphates in the cells;

where a decrease in the level of inositol phosphates in the cells in the presence of the suspected antagonist as compared to the level of inositol phosphates in the cells in the absence of the suspected antagonist indicates that the substance is an antagonist of HG03.

In a particular embodiment of the above-described method, the cells are eukaryotic cells. In another embodiment, the cells are mammalian cells. In other embodiments, the cells are L cells L-M(TK$^-$) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) and MRC-5 (ATCC CCL 171).

The conditions under which steps (b) and (c) of the method are practiced are conditions that are typically used in the art for the study of protein-ligand interactions: *e.g.*, physiological pH; salt conditions such as those represented by such commonly used buffers as PBS or in tissue culture media; a temperature of about 4°C to about 55°C.

In a particular embodiment of the above-described method, the cells are transfected with expression vectors that direct the expression of HG03 and the promiscuous G-protein in the cells.

In a particular embodiment of the above-described method, the promiscuous G-protein is selected from the group consisting of Gα15 or Gα16.

In particular embodiments of the above-described methods, HG03 has an amino acid sequence of SEQ.ID.NO.:2.

Another embodiment of the present invention are methods of expressing HG03 in recombinant systems and of identifying agonists and antagonists of HG03, and for counter-screening.

When screening compounds in order to identify potential pharmaceuticals that specifically interact with a target receptor, it is necessary to ensure that the compounds identified are as specific as possible for the target receptor. To do this, it is necessary to screen the compounds against as wide an array as possible of receptors that are similar to the target receptor. Thus, in order to find compounds that are

potential pharmaceuticals that interact with receptor A, it is necessary not only to ensure that the compounds interact with receptor A (the "plus target") and produce the desired pharmacological effect through receptor A, it is also necessary to determine that the compounds do not interact with receptors B, C, D, etc. (the "minus targets"). In general, as part of a screening program, it is important to have as many minus targets as possible (see Hodgson, 1992, Bio/Technology 10:973-980, at 980). HG03 proteins and DNA encoding HG03 proteins have utility in that they can be used as "minus targets" in screens design to identify compounds that specifically interact with other G-protein coupled receptors.

The present invention also includes antibodies to the HG03 protein. Such antibodies may be polyclonal antibodies or monoclonal antibodies. The antibodies of the present invention are raised against the entire HG03 protein or against suitable antigenic fragments of the protein that are coupled to suitable carriers, *e.g.*, serum albumin or keyhole limpet hemocyanin, by methods well known in the art. Methods of identifying suitable antigenic fragments of a protein are known in the art. See, *e.g.*, Hopp & Woods, 1981, Proc. Natl. Acad. Sci. USA 78:3824-3828; and Jameson & Wolf, 1988, CABIOS (Computer Applications in the Biosciences) 4:181-186.

For the production of polyclonal antibodies, HG03 protein or an antigenic fragment, coupled to a suitable carrier, is injected on a periodic basis into an appropriate non-human host animal such as, *e.g.*, rabbits, sheep, goats, rats, mice. The animals are bled periodically and sera obtained are tested for the presence of antibodies to the injected antigen. The injections can be intramuscular, intraperitoneal, subcutaneous, and the like, and can be accompanied with adjuvant.

For the production of monoclonal antibodies, HG03 protein or an antigenic fragment, coupled to a suitable carrier, is injected into an appropriate non-human host animal as above for the production of polyclonal antibodies. In the case of monoclonal antibodies, the animal is generally a mouse. The animal's spleen cells are then immortalized, often by fusion with a myeloma cell, as described in Kohler & Milstein, 1975, Nature 256:495-497. For a fuller description of the production of monoclonal antibodies, see Antibodies: A Laboratory Manual, Harlow & Lane, eds., Cold Spring Harbor Laboratory Press, 1988.

Gene therapy may be used to introduce HG03 polypeptides into the cells of target organs. Nucleotides encoding HG03 polypeptides can be ligated into viral vectors which mediate transfer of the nucleotides by infection of recipient cells. Suitable viral vectors include retrovirus, adenovirus, adeno-associated virus, herpes virus, vaccinia virus, and polio virus based vectors. Alternatively, nucleotides encoding HG03 polypeptides can be transferred into cells for gene therapy by non-viral techniques including receptor-mediated targeted transfer using ligand-nucleotide conjugates, lipofection, membrane fusion, or direct microinjection. These procedures and variations thereof are suitable for *ex vivo* as well as *in vivo* gene therapy. Gene therapy with HG03 polypeptides will be particularly useful for the treatment of diseases where it is beneficial to elevate HG03 activity.

The following non-limiting examples are presented to better illustrate the invention.

EXAMPLE 1

Cloning and sequencing of HG03

The full-length coding sequence of HG03 was isolated by multiple rounds of RCCA from a prostate cDNA library. Originally, the HG03 gene was named AOMF15 but was designated later HG03. The primer pair, F216 +R369 was initially used to scan the 1-2.5KB and 2.5KB prostate cDNA libraries. Positive pools were identified where nested insert-vector PCRs were then carried out using the following combinations of primers: first round PCR reactions, F216+543R, F216+873F, and R369+543R, R369+873F; nested second round PCR reaction, F325+578R, R280+383F. The list of primers used for the isolation of HG03:

AOMF15.F216	CCAGGCAAACATTACACGCA	(SEQ.ID.NO.:4)
AOMF15.F325	GAATTCTGCACAAGTGATACGGTA	(SEQ.ID.NO.:5)
AOMF15.R280	CAGGCTTTTAGATGAATCTGCA	(SEQ.ID.NO.:6)
AOMF15.R369	AGCATCAGGGTTGTTGTGGC	(SEQ.ID.NO.:7)
HG03.37R	GCGTGTCAGGAAACACTTGG	(SEQ.ID.NO.:8)
HG03_FL243F	AAAGAAATCAAACCAGGAATAACC	(SQ.ID.NO.:9)
HG03_FL1429R	CTTTGTACATATCGATTCCAACACAC	(SEQ.ID.NO.:10)

PBS.873F	CCCAGGCTTTACACTTTATGCTTCC	(SEQ.ID.NO.:11)
PBS.543R	GGGGATGTGCTGCAAGGCGA	(SEQ.ID.NO.:12)

PCR reactions were carried out with AmpliTaq (Perkin Elmer, CA and Taq extender (Stratagene) under the conditions suggested by the supplier of Taq extender. The PCR fragments were sequenced and assembled. Complete sequence was obtained by using primers 37R and 1118R by primer walking.

The analysis of this "race" PCR and sequencing reactions resulted in the assembly of a contiguous fragment of 1478 base pairs. This sequence contains an open reading frame of 358 amino acids. The full length coding sequence of HG03 was amplified by using primers FL-243F and FL-1429R and cloned into the plasmid vector pCR2.1 by TA cloning (Invitrogen, Carlsbad, CA). It is well within the skilled artisan to determine an appropriate vector for a particular gene transfer or other use.

EXAMPLE 2

Tissue distribution of HG03 RNA transcripts

An approximately 1.8 kb fragment containing HG03 was randomly labelled with ^{32}P-dCTP using the Megaprime DNA Labeling System (Amersham) and used to probe a Human MTN Blot (Clontech Cat # 7760-1, Clontech, Palo Alto, CA, USA). The MTN blot was hybridized in Expresshyb (Clontech) containing $2x10^6$ cpm/ml HG03 probe at 65°C overnight and washed to a final stringency of 0.1 X SSC/0.5% SDS at 65°C, and then exposed to X-ray film by autoradiography. The results are shown in Figure 4.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

WHAT IS CLAIMED:

1. A recombinant DNA molecule encoding a polypeptide having an amino acid sequence of SEQ.ID.NO.:2.

2. The DNA molecule of Claim 1 comprising a nucleotide sequence selected from the group consisting of SEQ.ID.NO.:1 and positions 346-1419 of SEQ.ID.NO.:1.

3. A DNA molecule that hybridizes under stringent conditions to the DNA of Claim 1.

4. An expression vector comprising the DNA of Claim 1.

5. A recombinant host cell comprising the DNA of Claim 1.

6. An HG03 protein, substantially free from other proteins, having an amino acid sequence of SEQ.ID.NO.:2.

7. The HG03 protein of Claim 6 containing a single amino acid substitution.

8. The HG03 protein of Claim 7 where the substitution is a conservative substitution.

9. The HG03 protein of Claim 6 containing two or more amino acid substitutions and substantially the same biological activity as HG03.

10. A method for determining whether a substance is capable of binding to HG03 comprising:

(a) providing test cells by transfecting cells with an expression vector that directs the expression of HG03 in the cells;

(b) exposing the test cells to the substance;

(c) measuring the amount of binding of the substance to HG03;

(d) comparing the amount of binding of the substance to HG03 in the test cells with the amount of binding of the substance to control cells that have not been transfected with HG03;

where HG03 has an amino acid sequence of SEQ.ID.NO.:2;

where if the amount of binding of the substance to HG03 in the test cells is greater than the amount of binding of the substance to the control cells, then the substance is capable of binding to HG03.

11. A method for determining whether a substance is capable of binding to HG03 comprising:

(a) providing test cells by transfecting cells with an expression vector that directs the expression of HG03 in the cells;

(b) preparing membranes containing HG03 from the test cells and exposing the membranes from the test cells to the substance;

(c) measuring the amount of binding of the substance to the HG03 in the membranes from the test cells;

(d) comparing the amount of binding of the substance to HG03 in the membranes from the test cells with the amount of binding of the substance to membranes from control cells that have not been transfected with HG03;

where HG03 has an amino acid sequence of SEQ.ID.NO.:2;

where if the amount of binding of the substance to HG03 in the membranes from the test cells is greater than the amount of binding of the substance to the membranes from the control cells, then the substance is capable of binding to HG03.

12. A method for determining whether a substance is capable of binding to HG03 comprising:

(a) providing test cells by transfecting cells with an expression vector that directs the expression of HG03 in the cells;

(b) exposing the test cells to a ligand of HG03 under conditions such that the ligand binds to the HG03 in the test cells;

(c) subsequently or concurrently to step (b), exposing the cells to a substance that is suspected of being capable of binding to HG03;

(d) measuring the amount of binding of the ligand to HG03 in the presence and the absence of the substance;

(e) comparing the amount of binding of the ligand to HG03 in the presence and the absence of the substance where a decrease in the amount of binding of the ligand to HG03 in the presence of the substance indicates that the substance is capable of binding to HG03;

where HG03 has an amino acid sequence of SEQ.ID.NO.:2.

13. A method for determining whether a substance is capable of binding to HG03 comprising:

(a) providing test cells by transfecting cells with an expression vector that directs the expression of HG03 in the cells;

(b) preparing membranes containing HG03 from the test cells and exposing the membranes to a ligand of HG03 under conditions such that the ligand binds to the HG03 in the membranes;

(c) subsequently or concurrently to step (b), exposing the membranes from the test cells to a substance;

(d) measuring the amount of binding of the ligand to the HG03 in the membranes in the presence and the absence of the substance;

(e) comparing the amount of binding of the ligand to HG03 in the membranes in the presence and the absence of the substance where a decrease in the amount of binding of the ligand to HG03 in the membranes in the presence of the substance indicates that the substance is capable of binding to HG03;

where HG03 has an amino acid sequence of SEQ.ID.NO.:2.

14. A method for determining whether a substance is a potential agonist or antagonist of HG03 that comprises:

(a) transfecting cells with an expression vector containing HG03;

(b) allowing the transfected cells to grow for a time sufficient to allow HG03 to be expressed;

(c) harvesting the transfected cells and resuspending the cells in assay buffer containing a known labeled agonist of HG03 in the presence and in the absence of the substance;

(d) measuring the binding of the known labeled agonist to HG03;

where if the amount of binding of the known labeled agonist is less in the presence of the substance than in the absence of the substance, then the substance is a potential agonist or antagonist of HG03;

where HG03 has an amino acid sequence of SEQ.ID.NO.:2.

15. A method of identifying agonists of HG03 comprising:

(a) providing cells that expresses both HG03 and a promiscuous G-protein;

(b) exposing the cells to a substance that is a suspected agonist of HG03;

(c) measuring the level of inositol phosphates in the cells;

where an increase in the level of inositol phosphates in the cells in the presence of the substance as compared to the level of inositol phosphates in the cells in the absence of the substance indicates that the substance is an agonist of HG03;

where HG03 has an amino acid sequence of SEQ.ID.NO.:2.

16. A method identifying antagonists of HG03 comprising:

(a) providing cells that expresses both HG03 and a promiscuous G-protein;

(b) exposing the cells to an agonist of HG03;

(c) subsequently or concurrently to step (b), exposing the cells to a substance that is a suspected antagonist of HG03;

(d) measuring the level of inositol phosphates in the cells;

where a decrease in the level of inositol phosphates in the cells in the presence of the substance as compared to the level of inositol phosphates in the cells in the absence of the substance indicates that the substance is an antagonist of HG03;

where HG03 has an amino acid sequence of SEQ.ID.NO.:2.

17. A method of identifying antagonists of HG03 comprising:

(a) providing cells that expresses a chimeric HG03 protein fused at its C-terminus to a promiscuous G-protein;

(b) exposing the cells to an agonist of HG03;

(c) subsequently or concurrently to step (b), exposing the cells to a substance that is a suspected antagonist of HG03;

(d) measuring the level of inositol phosphates in the cells;

where a decrease in the level of inositol phosphates in the cells in the presence of the substance as compared to the level of inositol phosphates in the cells in the absence of the substance indicates that the substance is an antagonist of HG03;

where the HG03 protein has an amino acid sequence of SEQ.ID.NO.:2.

18. An antibody that binds specifically to HG03 where HG03 has an amino acid sequence of SEQ.ID.NO.:2.

19. A method of expressing a truncated version of HG03 protein comprising:

(a) transfecting a host cell with a expression vector that encodes an HG03 protein that has been truncated at the amino terminus;

(b) culturing the transfected cells of step (a) under conditions such that the truncated HG03 protein is expressed.

20. The method of Claim 19 where the truncated HG03 protein is a chimeric HG03 protein.

FIG. 1 cDNA Polynucleotide sequence of HG03

```
   1  GCAAGTTGTT CCAGTAGTCG CCTGGCAGGA GAATTTGAAA GGGTGCCCCA
  51  AAGGACAATC TCTAAAGGGG TAAGGGAGAT ACCTACCTTG TCTGGTAGGG
 101  GAGATGTTTC GTTTTCATGC TTTACCAGAA AATCCACTTC CCTGCCGACC
 151  TTAGTTTCAA AGCTTATTCT TAATTAGAGA CAAGAAACCT GTTTCAACTT
 201  GAAGACACCG TATGAGGTGA ATGGACAGCC AGCCACCACA ATGAAAGAAA
 251  TCAAACCAGG AATAACCTAT GCTGAACCCA CGCCTCAATC GTCCCCAAGT
 301  GTTTCCTGAC ACGCATCTTT GCTTACAGTG CATCACAACT GAAGAATGGG
 351  GTTCAACTTG ACGCTTGCAA AATTACCAAA TAACGAGCTG CACGGCCAAG
 401  AGAGTCACAA TTCAGGCAAC AGGAGCGACG GGCCAGGAAA GAACACCACC
 451  CTTCACAATG AATTTGACAC AATTGTCTTG CCGGTGCTTT ATCTCATTAT
 501  ATTTGTGGCA AGCATCTTGC TGAATGGTTT AGCAGTGTGG ATCTTCTTCC
 551  ACATTAGGAA TAAAACCAGC TTCATATTCT ATCTCAAAAA CATAGTGGTT
 601  GCAGACCTCA TAATGACGCT GACATTTCCA TTTCGAATAG TCCATGATGC
 651  AGGATTTGGA CCTTGGTACT TCAAGTTTAT TCTCTGCAGA TACACTTCAG
 701  TTTTGTTTTA TGCAAACATG TATACTTCCA TCGTGTTCCT TGGGCTGATA
 751  AGCATTGATC GCTATCTGAA GGTGGTCAAG CCATTTGGGG ACTCTCGGAT
 801  GTACAGCATA ACCTTCACGA AGGTTTTATC TGTTTGTGTT TGGGTGATCA
 851  TGGCTGTTTT GTCTTTGCCA AACATCATCC TGACAAATGG TCAGCCAACA
 901  GAGGACAATA TCCATGACTG CTCAAAACTT AAAAGTCCTT TGGGGGTCAA
 951  ATGGCATACG GCAGTCACCT ATGTGAACAG CTGCTTGTTT GTGGCCGTGC
1001  TGGTGATTCT GATCGGATGT TACATAGCCA TATCCAGGTA CATCCACAAA
1051  TCCAGCAGGC AATTCATAAG TCAGTCAAGC CGAAAGCGAA AACATAACCA
1101  GAGCATCAGG GTTGTTGTGG CTGTGTTTTT TACCTGCTTT CTACCATATC
1151  ACTTGTGCAG AATTCCTTTT ACTTTTAGTC ACTTAGACAG GCTTTTAGAT
1201  GAATCTGCAC AAAAAATCCT ATATTACTGC AAAGAAATTA CACTTTTCTT
1251  GTCTGCGTGT AATGTTTGCC TGGATCCAAT AATTTACTTT TTCATGTGTA
1301  GGTCATTTTC AAGAAGGCTG TTCAAAAAAT CAAATATCAG AACCAGGAGT
1351  GAAAGCATCA GATCACTGCA AAGTGTGAGA AGATCGGAAG TTCGCATATA
1401  TTATGATTAC ACTGATGTGT AGGCCTTTTA TTGTGTGTTG GAATCGATAT
1451  GTACAAAGTG TAAATAAATG TTTCTTTT
```

FIG. 2. Predicted polypeptide sequence of HG03.

```
  1  MGFNLTLAKL PNNELHGQES HNSGNRSDGP GKNTTLHNEF DTIVLPVLYL
 51  IIFVASILLN GLAVWIFFHI RNKTSFIFYL KNIVVADLIM TLTFPFRIVH
101  DAGFGPWYFK FILCRYTSVL FYANMYTSIV FLGLISIDRY LKVVKPFGDS
151  RMYSITFTKV LSVCVWVIMA VLSLPNIILT NGQPTEDNIH DCSKLKSPLG
201  VKWHTAVTYV NSCLFVAVLV ILIGCYIAIS RYIHKSSRQF ISQSSRKRKH
251  NQSIRVVVAV FFTCFLPYHL CRIPFTFSHL DRLLDESAQK ILYYCKEITL
301  FLSACNVCLD PIIYFFMCRS FSRRLFKKSN IRTRSESIRS LQSVRRSEVR
351  IYYDYTDV*
```

FIG. 3A. Translation of The HG03 Open Reading Frame

```
        10                  30                  50
GCAAGTTGTTCCAGTAGTCGCCTGGCAGGAGAATTTGAAAGGGTGCCCCAAAGGACAATC

        70                  90                  110
TCTAAAGGGGTAAGGGAGATACCTACCTTGTCTGGTAGGGGAGATGTTTCGTTTTCATGC

       130                 150                 170
TTTACCAGAAAATCCACTTCCCTGCCGACCTTAGTTTCAAAGCTTATTCTTAATTAGAGA

       190                 210                 230
CAAGAAACCTGTTTCAACTTGAAGACACCGTATGAGGTGAATGGACAGCCAGCCACCACA

       250                 270                 290
ATGAAAGAAATCAAACCAGGAATAACCTATGCTGAACCCACGCCTCAATCGTCCCCAAGT

       310                 330                 350
GTTTCCTGACACGCATCTTTGCTTACAGTGCATCACAACTGAAGAATGGGGTTCAACTTG
                                                MetGlyPheAsnLeu

       370                 390                 410
ACGCTTGCAAAATTACCAAATAACGAGCTGCACGGCCAAGAGAGTCACAATTCAGGCAAC
ThrLeuAlaLysLeuProAsnAsnGluLeuHisGlyGlnGluSerHisAsnSerGlyAsn

       430                 450                 470
AGGAGCGACGGGCCAGGAAAGAACACCACCCTTCACAATGAATTTGACACAATTGTCTTG
ArgSerAspGlyProGlyLysAsnThrThrLeuHisAsnGluPheAspThrIleValLeu

       490                 510                 530
CCGGTGCTTTATCTCATTATATTTGTGGCAAGCATCTTGCTGAATGGTTTAGCAGTGTGG
ProValLeuTyrLeuIleIlePheValAlaSerIleLeuLeuAsnGlyLeuAlaValTrp

       550                 570                 590
ATCTTCTTCCACATTAGGAATAAAACCAGCTTCATATTCTATCTCAAAAACATAGTGGTT
IlePhePheHisIleArgAsnLysThrSerPheIlePheTyrLeuLysAsnIleValVal

       610                 630                 650
GCAGACCTCATAATGACGCTGACATTTCCATTTCGAATAGTCCATGATGCAGGATTTGGA
AlaAspLeuIleMetThrLeuThrPheProPheArgIleValHisAspAlaGlyPheGly
```

FIG. 3B. Translation of The HG03 Open Reading Frame

```
       670                   690                   710
CCTTGGTACTTCAAGTTTATTCTCTGCAGATACACTTCAGTTTTGTTTTATGCAAACATG
ProTrpTyrPheLysPheIleLeuCysArgTyrThrSerValLeuPheTyrAlaAsnMet

       730                   750                   770
TATACTTCCATCGTGTTCCTTGGGCTGATAAGCATTGATCGCTATCTGAAGGTGGTCAAG
TyrThrSerIleValPheLeuGlyLeuIleSerIleAspArgTyrLeuLysValValLys

       790                   810                   830
CCATTTGGGGACTCTCGGATGTACAGCATAACCTTCACGAAGGTTTTATCTGTTTGTGTT
ProPheGlyAspSerArgMetTyrSerIleThrPheThrLysValLeuSerValCysVal

       850                   870                   890
TGGGTGATCATGGCTGTTTTGTCTTTGCCAAACATCATCCTGACAAATGGTCAGCCAACA
TrpValIleMetAlaValLeuSerLeuProAsnIleIleLeuThrAsnGlyGlnProThr

       910                   930                   950
GAGGACAATATCCATGACTGCTCAAAACTTAAAAGTCCTTTGGGGGTCAAATGGCATACG
GluAspAsnIleHisAspCysSerLysLeuLysSerProLeuGlyValLysTrpHisThr

       970                   990                  1010
GCAGTCACCTATGTGAACAGCTGCTTGTTTGTGGCCGTGCTGGTGATTCTGATCGGATGT
AlaValThrTyrValAsnSerCysLeuPheValAlaValLeuValIleLeuIleGlyCys

      1030                  1050                  1070
TACATAGCCATATCCAGGTACATCCACAAATCCAGCAGGCAATTCATAAGTCAGTCAAGC
TyrIleAlaIleSerArgTyrIleHisLysSerSerArgGlnPheIleSerGlnSerSer

      1090                  1110                  1130
CGAAAGCGAAAACATAACCAGAGCATCAGGGTTGTTGTGGCTGTGTTTTTTACCTGCTTT
ArgLysArgLysHisAsnGlnSerIleArgValValValAlaValPhePheThrCysPhe

      1150                  1170                  1190
CTACCATATCACTTGTGCAGAATTCCTTTTACTTTTAGTCACTTAGACAGGCTTTTAGAT
LeuProTyrHisLeuCysArgIleProPheThrPheSerHisLeuAspArgLeuLeuAsp

      1210                  1230                  1250
GAATCTGCACAAAAAATCCTATATTACTGCAAAGAAATTACACTTTTCTTGTCTGCGTGT
GluSerAlaGlnLysIleLeuTyrTyrCysLysGluIleThrLeuPheLeuSerAlaCys
```

FIG. 3C. Translation of The HG03 Open Reading Frame

```
      1270                 1290                 1310
AATGTTTGCCTGGATCCAATAATTTACTTTTTCATGTGTAGGTCATTTTCAAGAAGGCTG
AsnValCysLeuAspProIleIleTyrPhePheMetCysArgSerPheSerArgArgLeu

      1330                 1350                 1370
TTCAAAAAATCAAATATCAGAACCAGGAGTGAAAGCATCAGATCACTGCAAAGTGTGAGA
PheLysLysSerAsnIleArgThrArgSerGluSerIleArgSerLeuGlnSerValArg

      1390                 1410                 1430
AGATCGGAAGTTCGCATATATTATGATTACACTGATGTGTAGGCCTTTTATTGTGTGTTG
ArgSerGluValArgIleTyrTyrAspTyrThrAspValEnd

      1450                 1470
GAATCGATATGTACAAAGTGTAAATAAATGTTTCTTTT
```

Fig. 4 . Multi-tissue Northern Analysis of HG03.

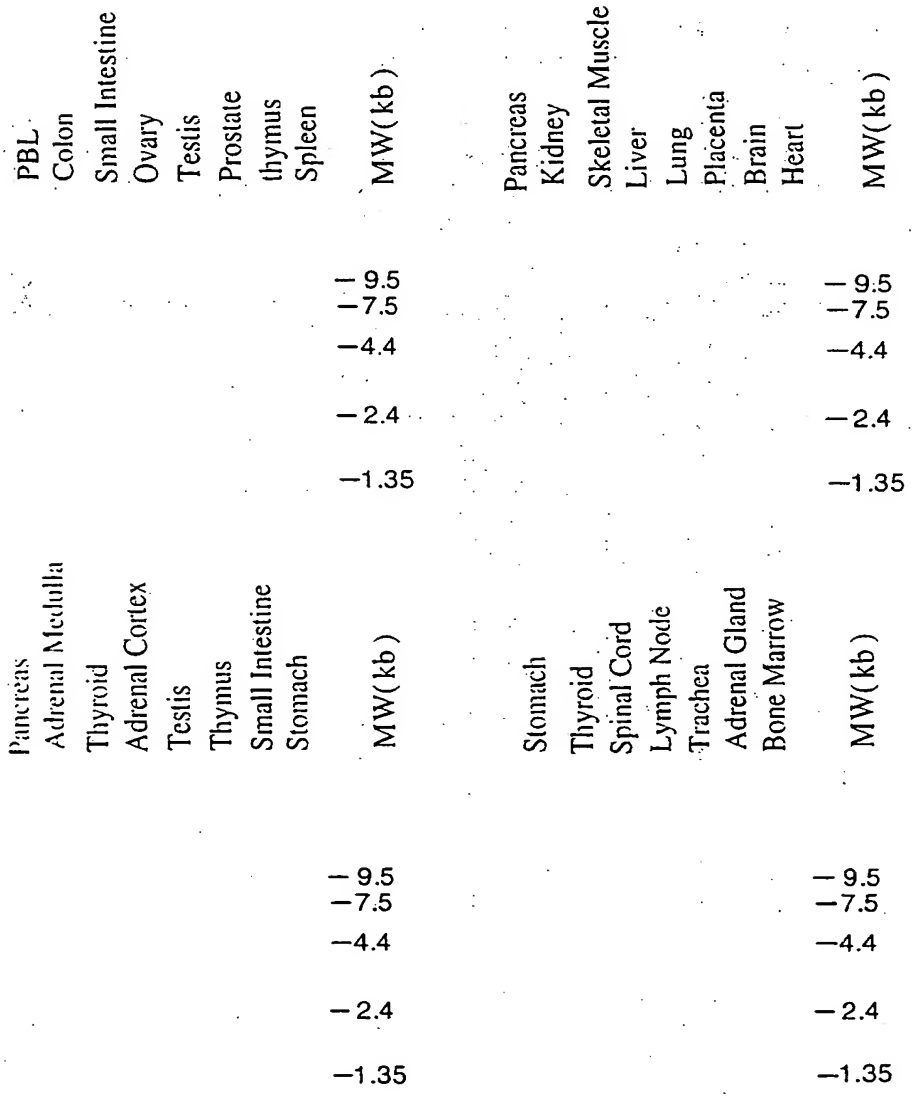

FIG. 5. Alignment of the polypeptide sequences of HG03 with that of the human platet activating factor receptor.

```
                                                  10        20        30
hPAFR.pep                                 MEPHDSSHMDSEFRYTLFPIVYSIIFVLGVIAN
                                                  |  ::  :  :||   ::|::| |||| :::  |
HG03          MGFNLTLAKLPNNELHGQESHNSGNRSDGPGKNTTLHNEFDTIVLPVLYLIIFVASILLN
                       10        20        30        40        50        60

                   40        50        60        70        80        90
hPAFR.pep     GYVLWVFARLYPCKKFNEIKIFMVNLTMADMLFLITLPLWIVYYQNQGNWILPKFLCNVA
              | ::|:| ::    :|   ::: |:::||::: :|:|: ||:   : | | :  :|| :
HG03          GLAVWIFFHIRNKTSFI---FYLKNIVVADLIMTLTFPFRIVHDAGFGPWYFKFILCRYT
                       70        80        90       100       110

                  100       110       120       130       140       150
hPAFR.pep     GCLFFINTYCSVAFLGVITYNRFQAVTRPIKTAQANTRKRGISLSLVIWVAIVGAASYFL
              : ||: | | |::|||:|: :|:  |::|:  ::  :       ||: :|| ::  :   :
HG03          SVLFYANMYTSIVFLGLISIDRYLKVVKPFGDSRMYSITFTKVLSVCVWVIMAVLSLPNI
              120       130       140       150       160       170

                  160       170       180       190       200       210
hPAFR.pep     ILDSTNTVPDSAGSGNVTRCFEHYEKGSVPVLIIHIFIVFSFFLVFLIILF-CNLVIIRT
              ||  ||  |       |: | :     :|       ::   :|:: |:||: | ::| |
HG03          IL--TNGQPTE---DNIHDCSKLKSPLGVKWHTAVTYVNSCLFVAVLVILIGCYIAISRY
                180       190       200       210       220       230

                  220       230       240       250       260       270
hPAFR.pep     LLMQPVQQQRNAEVKRRALWMVCTVLAVFIICFVPHHVVQLPWTLAELG-FQDSKFHQAI
              :  :  |   ::  ||:   : :|:|||: ||:|:|: ::|:|:::|  : | : :: :
HG03          IHKSSRQFISQSSRKRKHNQSIRVVVAVFFTCFLPYHLCRIPFTFSHLDRLLDESAQKIL
                  240       250       260       270       280       290

                  280       290       300       310       320       330
hPAFR.pep     NDAHQVTLCLLSTNCVLDPVIYCFLTKKFRKHLTEKFYSMRSSRKCSRATTDTVTEVVVP
                 :::|| | : |  |||:|| |: ::| ::| :|  ::|:  :  |:
HG03          YYCKEITLFLSACNVCLDPIIYFFMCRSFSRRLFKK-SNIRTRSESIRSLQSVRRSEVRI
                  300       310       320       330       340       350

                  340
hPAFR.pep     FNQIPGNSLKN

HG03          YYDYTDV
```

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/17388

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12N 5/10, 5/16, 15/12, 15/62, 15/63, 15/64; C07K 14/705

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.1, 69.7, 70.1, 71.1, 71.2, 471, 325, 252.3, 254.11, 320.1; 530/350; 536/23.1, 23.4, 23.5, 24.3, 24.31

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST search, CAS ONLINE, MEDLINE, EMBASE, BIOSIS, CAPLUS
search terms: receptor HG03, DNA, nucleic acid, expression, recombinant production, chimera, hybrid.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,144,007 A (PFAHL) 01 September 1992 (01/09/92), see entire document.	1-9, 19-20
A	US 4,985,352 A (JULIUS et al) 15 January 1991 (15/01/91), see entire document.	1-9, 19-20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 22 NOVEMBER 1999	Date of mailing of the international search report 03 DEC 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer PREMA MERTZ Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/17388

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-9, 19-20

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/17388

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/69.1, 70.1, 71.1, 71.2, 471, 325, 252.3, 254.11, 320.1; 530/350; 536/23.1, 23.5, 24.3, 24.31

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-9, 19-20, drawn to a DNA molecule encoding a G-protein coupled receptor HG03, an expression vector, host cell, G-protein coupled receptor HG03, and a method of expressing a truncated G-protein coupled receptor HG03.
Group II, claims 10-17, drawn to a method for determining whether a substance is capable of binding to G-protein coupled receptor HG03.
Group III, claim 18, drawn to an antibody that specifically binds G-protein coupled receptor HG03.
The inventions listed as Groups I-III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:
The inventions listed as Groups I-III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:
Pursuant to 37 C.F.R. § 1.475 (d), the ISA/US considers that where multiple products and processes are claimed, the main invention shall consist of the first invention of the category first mentioned in the claims and the first recited invention of each of the other categories related thereto. Accordingly, the main invention (Group I) comprises the first-recited product, a DNA molecule encoding a G-protein coupled receptor HG03, an expression vector, host cell, G-protein coupled receptor HG03, and a method of expressing a truncated G-protein coupled receptor HG03. Further pursuant to 37 C.F.R. § 1.475 (d), the ISA/US considers that any feature which the subsequently recited products and methods share with the main invention does not constitute a special technical feature within the meaning of PCT Rule 13.2 and that each of such products and methods accordingly defines a separate invention.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 February 2000 (17.02.2000)

PCT

(10) International Publication Number
WO 00/08133 A1

(51) International Patent Classification[6]: C12N 5/10, 5/16, 15/12, 15/62, 15/63, 15/64, C07K 14/705

(21) International Application Number: PCT/US99/17388

(22) International Filing Date: 2 August 1999 (02.08.1999)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/095,571 6 August 1998 (06.08.1998) US

(71) Applicant *(for all designated States except US)*: **MERCK & CO., INC.** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **LIU, Qingyun** [CN/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). **McDONALD, Terrence, P.** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). **WANG, Ruiping** [CN/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).

(74) Common Representative: **MERCK & CO., INC.**; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).

(81) Designated States *(national)*: CA, JP, US.

(84) Designated States *(regional)*: European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published:
— *with international search report*

(48) Date of publication of this corrected version:
20 December 2001

(15) Information about Correction:
see PCT Gazette No. 51/2001 of 20 December 2001, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/08133 A1

(54) Title: NOVEL G PROTEIN-COUPLED RECEPTOR cDNA SEQUENCE

(57) Abstract: cDNA encoding a novel human G-protein coupled receptor, HG03, as well as the protein encoded by the cDNA, is provided. Methods of identifying agonists and antagonists of HG03 are also provided.

TITLE OF THE INVENTION

NOVEL G PROTEIN-COUPLED RECEPTOR cDNA SEQUENCE

CROSS-REFERENCE TO RELATED APPLICATIONS

Not applicable.

STATEMENT REGARDING FEDERALLY-SPONSORED R&D

Not applicable.

REFERENCE TO MICROFICHE APPENDIX

Not applicable.

FIELD OF THE INVENTION

This invention relates to a novel human cDNA encoding a G protein-coupled receptor (GPCR) having homology to members of a family of receptors for nucleotides and platelet-activating factor, the protein encoded by the cDNA, and methods of identifying selective agonists and antagonists.

BACKGROUND OF THE INVENTION

G-protein coupled receptors (GPCRs) are a very large class of membrane receptors that relay information from the exterior to the interior of cells. GPCRs function by interacting with a class of heterotrimeric proteins known as G-proteins. Most GPCRs function by a similar mechanism. Upon the binding of agonist, a GPCR catalyzes the dissociation of guanosine diphosphate (GDP) from the α subunit of G proteins. This allows for the binding of guanosine triphosphate (GTP) to the α subunit, resulting in the disassociation of the α subunit from the β and γ subunits. The freed α subunit then interacts with other cellular components, and in the process passes on the extracellular signal represented by the presence of the agonist. Occasionally, it is the freed β and γ subunits which transduce the agonist signal.

GPCRs possess common structural characteristics. They have seven hydrophobic domains, about 20-30 amino acids long, linked by sequences of hydrophilic amino acids of varied length. These seven hydrophobic domains intercalate into the plasma membrane, giving rise to a protein with seven transmembrane domains, an extracellular amino terminus, and an intracellular

carboxy terminus (Strader et al., 1994, Ann. Rev. Biochem. 63:101-132; Schertler et al., 1993, Nature 362:770-7721; Dohlman et al., 1991, Ann. Rev. Biochem. 60:653-688).

GPCRs are expressed in a wide variety of tissue types and respond to a wide range of ligands, *e.g.*, protein hormones, biogenic amines, peptides, lipid derived messengers, etc. Given their wide range of expression and ligands, it is not surprising that GPCRs are involved in many pathological states. This has led to great interest in developing modulators of GPCR activity that can be used pharmacologically. For example, Table 1 of Stadel et al., 1997, Trends Pharmacol. Sci. 18:430-437, lists 37 different marketed drugs that act upon GPCRs. Accordingly, there is a great need to understand GPCR function and to develop agents that can be used to modulate GPCR activity.

SUMMARY OF THE INVENTION

The present invention is directed to a novel human cDNA that encodes a G-protein coupled receptor, HG03. The DNA encoding HG03 is substantially free from other nucleic acids and has the nucleotide sequence shown in SEQ.ID.NO.:1. Also provided is an HG03 protein encoded by the novel cDNA sequence. The HG03 protein is substantially free from other proteins and has the amino acid sequence shown in SEQ.ID.NO.:2. Methods of expressing HG03 in recombinant systems and of identifying agonists and antagonists of HG03 are provided.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the complete cDNA sequence of HG03 (SEQ.ID.NO.:1).

Figure 2 show the complete amino acid sequence of HG03 (SEQ.ID.:2).

Figure 3A-C shows the translation of HG03 open reading frame. The nucleotide sequence shown is (SEQ.ID.:1). The amino acid sequence shown is (SEQ.ID.:2).

Figure 4 shows the results of a Northern blot of the expression of HG03 mRNA in various human tissues.

Figure 5 shows the alignment of the amino acid sequence of HG03 with the amino acid sequence of the human platelet activating factor receptor (SEQ.ID.:3).

DETAILED DESCRIPTION OF THE INVENTION

For the purposes of this invention:

"Substantially free from other proteins" means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other proteins. Thus, an HG03 protein preparation that is substantially free from other proteins will contain, as a percent of its total protein, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non-HG03 proteins. Whether a given HG03 protein preparation is substantially free from other proteins can be determined by such conventional techniques of assessing protein purity as, *e.g.*, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) combined with appropriate detection methods, *e.g.*, silver staining or immunoblotting.

"Substantially free from other nucleic acids" means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other nucleic acids. Thus, an HG03 DNA preparation that is substantially free from other nucleic acids will contain, as a percent of its total nucleic acid, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non-HG03 nucleic acids. Whether a given HG03 DNA preparation is substantially free from other nucleic acids can be determined by such conventional techniques of assessing nucleic acid purity as, *e.g.*, agarose gel electrophoresis combined with appropriate staining methods, *e.g.*, ethidium bromide staining, or by sequencing.

"Functional equivalent" means a receptor which does not have the exact same amino acid sequence of a naturally occurring G protein-coupled receptor, due to alternative splicing, deletions, mutations, or additions, but retains at least 1%, preferably 10%, and more preferably 25% of the biological activity of the naturally occurring receptor. Such derivatives will have a significant homology with a natural GPCR and genes and cDNA encoding such derivatives can be detected by reduced stringency hybridization with a DNA sequence encoding a natural GPCR. The nucleic acid encoding a functional equivalent has at least about 50% homology at the nucleotide level to a naturally occurring receptor nucleic acid.

A polypeptide has "substantially the same biological activity" as HG03 if that polypeptide has a K_d for a ligand that is no more than 5-fold greater than the K_d of HG03 for the same ligand.

A "conservative amino acid substitution" refers to the replacement of one amino acid residue by another, chemically similar, amino acid residue. Examples of such conservative substitutions are: substitution of one hydrophobic residue (isoleucine, leucine, valine, or methionine) for another; substitution of one polar residue for another polar residue of the same charge (*e.g.*, arginine for lysine; glutamic acid for aspartic acid).

One aspect of this invention is the identification and cloning of a novel G protein-coupled receptor (GPCR), substantially free from receptor associated proteins, designated as HG03.

Another aspect of this invention is nucleic acids which encode the HG03 G protein-coupled receptor. These nucleic acids are substantially free from associated nucleic acids. For most cloning purposes, DNA is a preferred nucleic acid.

The present invention provides a cDNA molecule substantially free from other nucleic acids having the nucleotide sequence shown in FIGURE 1 as SEQ.ID.NO.:1. Analysis of FIGURE 3A-C revealed that SEQ.ID.NO:1 contains an open reading frame at positions 346-1419. Thus, the present invention also provides a cDNA molecule substantially free from other nucleic acids comprising the nucleotide sequence of positions 346-1419 of SEQ.ID.NO.:1. The present invention also provides recombinant DNA molecules comprising the nucleotide sequence of positions 346-1419 of SEQ.ID.NO.:1.

Sequence analysis of the open reading frame of the HG03 cDNA revealed that it encodes a protein of 358 amino acids. Based on its predicted amino acid sequence, HG03 most likely represents a novel GPCR. Northern blot analysis showed that HG03 RNA is highly expressed in the prostate, placenta, and trachea in human with a major transcript of ~1.8 kb and a minor transcript of ~8.0 kb. HG03 was also expressed at lower levels in thymus and testis as a transcript of ~1.8 kb. HG03 appears to be related to the members of receptors for nucleotides and platelet-activating factor.

The novel DNA sequences of the present invention encoding HG03, in whole or in part, can be linked with other DNA sequences, *i.e.*, DNA sequences to which HG03 is not naturally linked, to form "recombinant DNA molecules" containing HG03. The novel DNA sequences of the present invention can be inserted into vectors which comprise nucleic acids encoding a GPCR or a functional equivalent. These vectors may be comprised of DNA or RNA; for most cloning purposes DNA vectors are preferred. Typical vectors include plasmids, modified viruses, bacteriophage and cosmids, yeast artificial chromosomes and other forms of episomal or integrated DNA that can encode a GPCR. It is well within the skilled artisan to determine an appropriate vector for a particular gene transfer or other use.

Included in the present invention are cDNA sequences that hybridize to SEQ.ID.NO.:1 under stringent conditions. By way of example, and not limitation, a procedure using conditions of high stringency is as follows: Prehybridization of filters containing DNA is carried out for 2 hr. to overnight at 65°C in buffer composed of 6X SSC, 5X Denhardt's solution, and 100 µg/ml denatured salmon sperm DNA. Filters are hybridized for 12 to 48 hrs at 65°C in prehybridization mixture containing 100 µg/ml denatured salmon sperm DNA and 5-20 X 10^6 cpm of ^{32}P-labeled probe. Washing of filters is done at 37°C for 1 hr in a solution containing 2X SSC, 0.1% SDS. This is followed by a wash in 0.1X SSC, 0.1% SDS at 50°C for 45 min. before autoradiography.

Other procedures using conditions of high stringency would include either a hybridization step carried out in 5XSSC, 5X Denhardt's solution, 50% formamide at 42°C for 12 to 48 hours or a washing step carried out in 0.2X SSPE, 0.2% SDS at 65°C for 30 to 60 minutes.

Reagents mentioned in the foregoing procedures for carrying out high stringency hybridization are well known in the art. Details of the composition of these reagents can be found in, *e.g.*, Sambrook, Fritsch, and Maniatis, 1989, Molecular Cloning: A Laboratory Manual, second edition, Cold Spring Harbor Laboratory Press. In addition to the foregoing, other conditions of high stringency which may be used are well known in the art.

Another aspect of the present invention includes host cells that have been engineered to contain and/or express DNA sequences encoding HG03. Such

recombinant host cells can be cultured under suitable conditions to produce HG03. An expression vector containing DNA encoding HG03 can be used for expression of HG03 in a recombinant host cell. Recombinant host cells may be prokaryotic or eukaryotic, including but not limited to, bacteria such as *E. coli*, fungal cells such as yeast, mammalian cells including, but not limited to, cell lines of human, bovine, porcine, monkey and rodent origin, and insect cells including but not limited to *Drosophila* and silkworm derived cell lines. Cell lines derived from mammalian species which are suitable for recombinant expression of HG03 and which are commercially available, include but are not limited to, L cells L-M(TK$^-$) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) and MRC-5 (ATCC CCL 171).

Human embryonic kidney (HEK 293) cells and Chinese hamster ovary (CHO) cells are particularly suitable for expression of the HG03 protein because these cells express a large number of G-proteins. Thus, it is likely that at least one of these G-proteins will be able to functionally couple the signal generated by interaction of HG03 and its ligands, thus transmitting this signal to downstream effectors, eventually resulting in a measurable change in some assayable component, *e.g.*, cAMP level, expression of a reporter gene, hydrolysis of inositol lipids, or intracellular Ca^{2+} levels.

A variety of mammalian expression vectors can be used to express recombinant HG03 in mammalian cells. Commercially available mammalian expression vectors which are suitable include, but are not limited to, pCR2.1 (Invitrogen), pMC1neo (Stratagene), pSG5 (Stratagene), pcDNAI and pcDNAIamp, pcDNA3, pcDNA3.1, pCR3.1 (Invitrogen), EBO-pSV2-neo (ATCC 37593), pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), and pSV2-dhfr (ATCC 37146). Following expression in recombinant cells, HG03 can be purified by conventional techniques to a level that is substantially free from other proteins.

As with many receptor proteins, it is possible to modify many of the amino acids of HG03, particularly those which are not found in the ligand binding domain, and still retain substantially the same biological activity as the original receptor. Thus this invention includes modified HG03 polypeptides which have

amino acid deletions, additions, or substitutions but that still retain substantially the same biological activity as HG03. It is generally accepted that single amino acid substitutions do not usually alter the biological activity of a protein (see, *e.g.*, Molecular Biology of the Gene, Watson *et al.*, 1987, Fourth Ed., The Benjamin/Cummings Publishing Co., Inc., page 226; and Cunningham & Wells, 1989, Science 244:1081-1085). Accordingly, the present invention includes polypeptides where one amino acid substitution has been made in SEQ.ID.NO.:2 wherein the polypeptides still retain substantially the same biological activity as HG03. The present invention also includes polypeptides where two or more amino acid substitutions have been made in SEQ.ID.NO.:2 wherein the polypeptides still retain substantially the same biological activity as HG03. In particular, the present invention includes embodiments where the above-described substitutions are conservative substitutions. In particular, the present invention includes embodiments where the above-described substitutions do not occur in the ligand-binding domain of HG03.

The present invention also includes C-terminal truncated forms of HG03, particularly those which encompass the extracellular portion of the receptor, but lack the intracellular signaling portion of the receptor. Such truncated receptors are useful in various binding assays described herein, for crystallization studies, and for structure-activity-relationship studies.

Romano *et al.*, 1996, J. Biol. Chem. 271:28612-28616 demonstrated that some GPCRs are often found as homodimers formed by an intermolecular disulfide bond. The location of the cysteines responsible for the disulfide bond was found to be in the amino terminal 17 kd of the receptors. Accordingly, the present invention includes dimers of HG03 proteins.

It has been found that, in some case, membrane spanning regions of receptor proteins can be used to inhibit receptor function (Ng *et al.*, 1996, Biochem. Biophys. Res. Comm. 227:200-204; Hebert *et al.*, 1996, J. Biol. Chem. 271, 16384-16392; Lofts *et al.*, Oncogene 8:2813-2820). Accordingly, the present invention provides peptides derived from the seven membrane spanning regions of HG03 and their use to inhibit HG03 function. Such peptides can include the whole or parts of the receptor membrane spanning domains.

The present invention also includes chimeric HG03 proteins. Chimeric HG03 proteins consist of a contiguous polypeptide sequence of HG03 fused in frame

to a polypeptide sequence of a non-HG03 protein. For example, the N-terminal domain and seven transmembrane spanning domains of HG03 fused at the C-terminus in frame to a G protein would be a chimeric HG03 protein.

The present invention also includes HG03 proteins that are in the form of multimeric structures, *e.g.*, dimers. Such multimers of other G-protein coupled receptors are known (Hebert *et al.*, 1996, J. Biol. Chem. 271, 16384-16392; Ng *et al.*, 1996, Biochem. Biophys. Res. Comm. 227, 200-204; Romano *et al.*, 1996, J. Biol. Chem. 271, 28612-28616).

The present invention also includes isolated forms of HG03 proteins. By "isolated HG03 protein" is meant HG03 protein that has been isolated from a natural source. Use of the term "isolated" indicates that HG03 protein has been removed from its normal cellular environment. Thus, an isolated HG03 protein may be in a cell-free solution or placed in a different cellular environment from that in which it occurs naturally. The term isolated does not imply that an isolated HG03 protein is the only protein present, but instead means that an isolated HG03 protein is at least 95% free of non-amino acid material (*e.g.*, nucleic acids, lipids, carbohydrates) naturally associated with the HG03 protein. Thus, an HG03 protein that is expressed in bacteria or even in eukaryotic cells which do not naturally (*i.e.*, without human intervention) express it through recombinant means is an "isolated HG03 protein."

The specificity of binding of compounds showing affinity for HG03 is shown by measuring the affinity of the compounds for recombinant cells expressing the cloned receptor or for membranes from these cells. Expression of the cloned receptor and screening for compounds that bind to HG03 or that inhibit the binding of a known, radiolabeled ligand of HG03 to these cells, or membranes prepared from these cells, provides an effective method for the rapid selection of compounds with high affinity for HG03. Such ligands need not necessarily be radiolabeled but can also be nonisotopic compounds that can be used to displace bound radiolabeled compounds or that can be used as activators in functional assays. Compounds identified by the above method are likely to be agonists or antagonists of HG03 and may be peptides, proteins, or non-proteinaceous organic molecules.

Therefore, the present invention include assays by which HG03 agonists and antagonists may be identified. Methods for identifying agonists and antagonists of other receptors are well known in the art and can be adapted to identify agonists and antagonists of HG03. For

example, Cascieri *et al.*, 1992, Molec. Pharmacol. 41:1096-1099 describe a method for identifying substances that inhibit agonist binding to rat neurokinin receptors and thus are potential agonists or antagonists of neurokinin receptors. The method involves transfecting COS cells with expression vectors containing rat neurokinin receptors, allowing the transfected cells to grow for a time sufficient to allow the neurokinin receptors to be expressed, harvesting the transfected cells and resuspending the cells in assay buffer containing a known radioactively labeled agonist of the neurokinin receptors either in the presence or the absence of the substance, and then measuring the binding of the radioactively labeled known agonist of the neurokinin receptor to the neurokinin receptor. If the amount of binding of the known agonist is less in the presence of the substance than in the absence of the substance, then the substance is a potential agonist or antagonist of the neurokinin receptor.

Accordingly, the present invention includes a method for determining whether a substance is a potential agonist or antagonist of HG03 that comprises:

(a) transfecting cells with an expression vector encoding HG03;

(b) allowing the transfected cells to grow for a time sufficient to allow HG03 to be expressed;

(c) harvesting the transfected cells and resuspending the cells in the presence of a known labeled agonist of HG03 in the presence and in the absence of the substance;

(d) measuring the binding of the labeled agonist to HG03;

where if the amount of binding of the known agonist is less in the presence of the substance than in the absence of the substance, then the substance is a potential agonist or antagonist of HG03.

In a modification of the above-described method, step (b) is modified in that the cells are stably transfected with the expression vector containing HG03. In another modification of the above-described method, step (c) is modified in that the cells are not harvested and resuspended but rather the radioactively labeled known agonist and the substance are

contacted with the cells while the cells are attached to a substratum, *e.g.*, tissue culture plates.

The conditions under which step (c) of the method is practiced are conditions that are typically used in the art for the study of protein-ligand interactions: *e.g.*, physiological pH; salt conditions such as those represented by such commonly used buffers as PBS or in tissue culture media; a temperature of about 4°C to about 55°C.

In a particular embodiment of the above-described method, the cells are eukaryotic cells. In another embodiment, the cells are mammalian cells. In other embodiments, the cells are L cells L-M(TK$^-$) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) or MRC-5 (ATCC CCL 171).

The present invention also includes a method for determining whether a substance is capable of binding to HG03, *i.e.*, whether the substance is a potential agonist or an antagonist of HG03, where the method comprises:

(a) providing test cells by transfecting cells with an expression vector that directs the expression of HG03 in the cells;

(b) exposing the test cells to the substance;

(c) measuring the amount of binding of the substance to HG03;

(d) comparing the amount of binding of the substance to HG03 in the test cells with the amount of binding of the substance to control cells that have not been transfected with HG03;

wherein if the amount of binding of the substance is greater in the test cells as compared to the control cells, the substance is capable of binding to HG03. Determining whether the substance is actually an agonist or antagonist can then be accomplished by the use of functional assays such as, *e.g.*, the assay involving the use of promiscuous G-proteins described below.

The conditions under which step (b) of the method is practiced are conditions that are typically used in the art for the study of protein-ligand interactions: *e.g.*, physiological pH; salt conditions such as those represented by such commonly used buffers as PBS or in tissue culture media; a temperature of about 4°C to about 55°C.

The assays described above can be carried out with cells that have been transiently or stably transfected with HG03. Transfection is meant to include any method known in the art for introducing HG03 into the test cells. For example, transfection includes calcium phosphate or calcium chloride mediated transfection, lipofection, infection with a retroviral construct containing HG03, and electroporation.

Where binding of the substance or agonist to HG03 is measured, such binding can be measured by employing a labeled substance or agonist. The substance or agonist can be labeled in any convenient manner known to the art, *e.g.*, radioactively, fluorescently, enzymatically.

In particular embodiments of the above-described methods, HG03 has an amino acid sequence of SEQ.ID.NO.:2.

The above-described methods can be modified in that, rather than exposing the test cells to the substance, membranes can be prepared from the test cells and those membranes can be exposed to the substance. Such a modification utilizing membranes rather than cells is well known in the art and is described in, *e.g.*, Hess *et al.*, 1992, Biochem. Biophys. Res. Comm. 184:260-268.

Accordingly, the present invention provides a method for determining whether a substance is capable of binding to HG03 comprising:

(a) providing test cells by transfecting cells with an expression vector that directs the expression of HG03 in the cells;

(b) preparing membranes containing HG03 from the test cells and exposing the membranes from the test cells to the substance;

(c) measuring the amount of binding of the substance to the HG03 in the membranes from the test cells;

(d) comparing the amount of binding of the substance to HG03 in the membranes from the test cells with the amount of binding of the substance to membranes from control cells that have not been transfected with HG03;

where HG03 has an amino acid sequence of SEQ.ID.NO.:2;

where if the amount of binding of the substance to HG03 in the membranes from the test cells is greater than the amount of binding of the substance to the membranes from the control cells, then the substance is capable of binding to HG03.

The present invention provides a method for determining whether a substance is capable of binding to HG03 comprising:

(a) providing test cells by transfecting cells with an expression vector that directs the expression of HG03 in the cells;

(b) exposing the test cells to a ligand of HG03 under conditions such that the ligand binds to the HG03 in the test cells;

(c) subsequently or concurrently to step (b), exposing the cells to a substance that is suspected of being capable of binding to HG03;

(d) measuring the amount of binding of the ligand to HG03 in the presence and the absence of the substance;

(e) comparing the amount of binding of the ligand to HG03 in the presence and the absence of the substance where a decrease in the amount of binding of the ligand to HG03 in the presence of the substance indicates that the substance is capable of binding to HG03;

where HG03 has an amino acid sequence of SEQ.ID.NO.:2.

The present invention provides a method for determining whether a substance is capable of binding to HG03 comprising:

(a) providing test cells by transfecting cells with an expression vector that directs the expression of HG03 in the cells;

(b) preparing membranes containing HG03 from the test cells and exposing the membranes to a ligand of HG03 under conditions such that the ligand binds to the HG03 in the membranes;

(c) subsequently or concurrently to step (b), exposing the membranes from the test cells to a substance;

(d) measuring the amount of binding of the ligand to the HG03 in the membranes in the presence and the absence of the substance;

(e) comparing the amount of binding of the ligand to HG03 in the membranes in the presence and the absence of the substance where a decrease in the amount of binding of the ligand to HG03 in the membranes in the presence of the substance indicates that the substance is capable of binding to HG03;

where HG03 has an amino acid sequence of SEQ.ID.NO.:2.

As a further modification of the above-described methods, RNA encoding HG03 can be prepared as, *e.g.*, by *in vitro* transcription using a plasmid containing HG03 under the control of a bacteriophage T7 promoter, and the RNA can be microinjected into *Xenopus* oocytes in order to cause the expression of HG03 in the oocytes. Substances are then tested for binding to the HG03 expressed in the oocytes. Alternatively, rather than detecting binding, the effect of the substances on the electrophysiological properties of the oocytes can be determined.

The present invention includes assays by which HG03 agonists and antagonists may be identified by their ability to stimulate or antagonize a functional response mediated by HG03. HG03 belongs to the class of proteins known as G-protein coupled receptors (GPCRs). GPCRs transmit signals across cell membranes upon the binding of ligand. The ligand-bound GPCR interacts with a heterotrimeric G-protein, causing the Gα subunit of the G-protein to disassociate from the Gβ and Gγ subunits. The Gα subunit can then go on to activate a variety of second messenger systems.

Generally, a particular GPCR is only coupled to a particular type of G-protein. Thus, to observe a functional response from the GPCR, it is necessary to ensure that the proper G-protein is present in the system containing the GPCR. It has been found, however, that there are certain G-proteins that are "promiscuous." These promiscuous G-proteins will couple to, and thus transduce a functional signal from, virtually any GPCR. See Offermanns & Simon, 1995, J. Biol. Chem. 270:15175, 15180 (Offermanns). Offermanns described a system in which cells are transfected with expression vectors that result in the expression of one of a large number of GPCRs as well as the expression of one of the promiscuous G-proteins Gα15 or Gα16. Upon the addition of an agonist of the GPCR to the transfected cells, the GPCR was activated and was able,

via Gα15 or Gα16, to activate the β isoform of phospholipase C, leading to an increase in inositol phosphate levels in the cells.

Therefore, by making use of these promiscuous G-proteins as in Offermanns, it is possible to set up functional assays for HG03, even in the absence of knowledge of the G-protein with which HG03 is coupled *in vivo*. One possibility is to create a fusion or chimeric protein composed of the extracellular and membrane spanning portion of HG03 fused to a promiscuous G-protein. Such a fusion protein would be expected to transduce a signal following binding of ligand to the HG03 portion of the fusion protein. Accordingly, the present invention provides a method of identifying antagonists of HG03 comprising:

(a) providing cells that expresses a chimeric HG03 protein fused at its C-terminus to a promiscuous G-protein;

(b) exposing the cells to an agonist of HG03;

(c) subsequently or concurrently to step (b), exposing the cells to a substance that is a suspected antagonist of HG03;

(d) measuring the level of inositol phosphates in the cells;

where a decrease in the level of inositol phosphates in the cells in the presence of the substance as compared to the level of inositol phosphates in the cells in the absence of the substance indicates that the substance is an antagonist of HG03.

Another possibility for utilizing promiscuous G-proteins in connection with HG03 includes a method of identifying agonists of HG03 comprising:

(a) providing cells that expresses both HG03 and a promiscuous G-protein;

(b) exposing the cells to a substance that is a suspected agonist of HG03;

(c) measuring the level of inositol phosphates in the cells;

where an increase in the level of inositol phosphates in the cells as compared to the level of inositol phosphates in the cells in the absence of the suspected agonist ~~indicates that the~~ substance is an agonist of HG03.

Levels of inositol phosphates can be measured by monitoring calcium mobilization. Intracellular calcium mobilization is typically

assayed in whole cells under a microscope using fluorescent dyes or in cell suspensions via luminescence using the aequorin assay.

In a particular embodiment of the above-described method, the cells are eukaryotic cells. In another embodiment, the cells are mammalian cells. In other embodiments, the cells are L cells L-M(TK$^-$) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) or MRC-5 (ATCC CCL 171).

In a particular embodiment of the above-described method, the cells are transfected with expression vectors that direct the expression of HG03 and the promiscuous G-protein in the cells.

The conditions under which step (b) of the method is practiced are conditions that are typically used in the art for the study of protein-ligand interactions: *e.g.*, physiological pH; salt conditions such as those represented by such commonly used buffers as PBS or in tissue culture media; a temperature of about 4°C to about 55°C.

In a particular embodiment of the above-described method, the promiscuous G-protein is selected from the group consisting of Gα15 or Gα16. Expression vectors containing Gα15 or Gα16 are known in the art. See, *e.g.*, Offermanns; Buhl *et al.*, 1993, FEBS Lett. 323:132-134; Amatruda *et al.*, 1993, J. Biol. Chem. 268:10139-10144.

The above-described assay can be easily modified to form a method to identify antagonists of HG03. Such a method is also part of the present invention and comprises:

(a) providing cells that expresses both HG03 and a promiscuous G-protein;

(b) exposing the cells to a substance that is an agonist of HG03;

(c) subsequently or ~~concurrently to step (b),~~ exposing the cells to a substance that is a suspected antagonist of HG03;

(d) measuring the level of inositol phosphates in the cells;

where a decrease in the level of inositol phosphates in the cells in the presence of the suspected antagonist as compared to the level of inositol phosphates in the cells in the absence of the suspected antagonist indicates that the substance is an antagonist of HG03.

In a particular embodiment of the above-described method, the cells are eukaryotic cells. In another embodiment, the cells are mammalian cells. In other embodiments, the cells are L cells L-M(TK-) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70); COS-1 (ATCC CRL 1650); COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) and MRC-5 (ATCC CCL 171).

The conditions under which steps (b) and (c) of the method are practiced are conditions that are typically used in the art for the study of protein-ligand interactions: *e.g.*, physiological pH; salt conditions such as those represented by such commonly used buffers as PBS or in tissue culture media; a temperature of about 4°C to about 55°C.

In a particular embodiment of the above-described method, the cells are transfected with expression vectors that direct the expression of HG03 and the promiscuous G-protein in the cells.

In a particular embodiment of the above-described method, the promiscuous G-protein is selected from the group consisting of Gα15 or Gα16.

In particular embodiments of the above-described methods, HG03 has an amino acid sequence of SEQ.ID.NO.:2.

Another embodiment of the present invention are methods of expressing HG03 in recombinant systems and of identifying agonists and antagonists of HG03, and for counter-screening.

When screening compounds in order to identify potential pharmaceuticals that specifically interact with a target receptor, it is necessary to ensure that the compounds identified are as specific as possible for the target receptor. To do this, it is necessary to screen the compounds against as wide an array as possible of receptors that are similar to the target receptor. Thus, in order to find compounds that are

potential pharmaceuticals that interact with receptor A, it is necessary not only to ensure that the compounds interact with receptor A (the "plus target") and produce the desired pharmacological effect through receptor A, it is also necessary to determine that the compounds do not interact with receptors B, C, D, etc. (the "minus targets"). In general, as part of a screening program, it is important to have as many minus targets as possible (see Hodgson, 1992, Bio/Technology 10:973-980, at 980). HG03 proteins and DNA encoding HG03 proteins have utility in that they can be used as "minus targets" in screens design to identify compounds that specifically interact with other G-protein coupled receptors.

The present invention also includes antibodies to the HG03 protein. Such antibodies may be polyclonal antibodies or monoclonal antibodies. The antibodies of the present invention are raised against the entire HG03 protein or against suitable antigenic fragments of the protein that are coupled to suitable carriers, *e.g.*, serum albumin or keyhole limpet hemocyanin, by methods well known in the art. Methods of identifying suitable antigenic fragments of a protein are known in the art. See, *e.g.*, Hopp & Woods, 1981, Proc. Natl. Acad. Sci. USA 78:3824-3828; and Jameson & Wolf, 1988, CABIOS (Computer Applications in the Biosciences) 4:181-186.

For the production of polyclonal antibodies, HG03 protein or an antigenic fragment, coupled to a suitable carrier, is injected on a periodic basis into an appropriate non-human host animal such as, *e.g.*, rabbits, sheep, goats, rats, mice. The animals are bled periodically and sera obtained are tested for the presence of antibodies to the injected antigen. The injections can be intramuscular, intraperitoneal, subcutaneous, and the like, and can be accompanied with adjuvant.

For the production of monoclonal antibodies, HG03 protein or an antigenic fragment, coupled to a suitable carrier, is injected into an appropriate non-human host animal as above for the production of polyclonal antibodies. In the case of monoclonal antibodies, the animal is generally a mouse. The animal's spleen cells are then immortalized, often by fusion with a myeloma cell, as described in Kohler & Milstein, 1975, Nature 256:495-497. For a fuller description of the production of monoclonal antibodies, see Antibodies: A Laboratory Manual, Harlow & Lane, eds., Cold Spring Harbor Laboratory Press, 1988.

Gene therapy may be used to introduce HG03 polypeptides into the cells of target organs. Nucleotides encoding HG03 polypeptides can be ligated into viral vectors which mediate transfer of the nucleotides by infection of recipient cells. Suitable viral vectors include retrovirus, adenovirus, adeno-associated virus, herpes virus, vaccinia virus, and polio virus based vectors. Alternatively, nucleotides encoding HG03 polypeptides can be transferred into cells for gene therapy by non-viral techniques including receptor-mediated targeted transfer using ligand-nucleotide conjugates, lipofection, membrane fusion, or direct microinjection. These procedures and variations thereof are suitable for *ex vivo* as well as *in vivo* gene therapy. Gene therapy with HG03 polypeptides will be particularly useful for the treatment of diseases where it is beneficial to elevate HG03 activity.

The following non-limiting examples are presented to better illustrate the invention.

EXAMPLE 1

Cloning and sequencing of HG03

The full-length coding sequence of HG03 was isolated by multiple rounds of RCCA from a prostate cDNA library. Originally, the HG03 gene was named AOMF15 but was designated later HG03. The primer pair, F216 +R369 was initially used to scan the 1-2.5KB and 2.5KB prostate cDNA libraries. Positive pools were identified where nested insert-vector PCRs were then carried out using the following combinations of primers: first round PCR reactions, F216+543R, F216+873F, and R369+543R, R369+873F; nested second round PCR reaction, F325+578R, R280+383F. The list of primers used for the isolation of HG03:

AOMF15.F216	CCAGGCAAACATTACACGCA	(SEQ.ID.NO.:4)
AOMF15.F325	GAATTCTGCACAAGTGATACGGTA	(SEQ.ID.NO.:5)
AOMF15.R280	CAGGCTTTTAGATGAATCTGCA	(SEQ.ID.NO.:6)
AOMF15.R369	AGCATCAGGGTTGTTGTGGC	(SEQ.ID.NO.:7)
HG03.37R	GCGTGTCAGGAAACACTTGG	(SEQ.ID.NO.:8)
HG03_FL243F	AAAGAAATCAAACCAGGAATAACC	(SQ.ID.NO.:9)
HG03_FL1429R	CTTTGTACATATCGATTCCAACACAC	(SEQ.ID.NO.:10)

PBS.873F	CCCAGGCTTTACACTTTATGCTTCC	(SEQ.ID.NO.:11)
PBS.543R	GGGGATGTGCTGCAAGGCGA	(SEQ.ID.NO.:12)

PCR reactions were carried out with AmpliTaq (Perkin Elmer, CA and Taq extender (Stratagene) under the conditions suggested by the supplier of Taq extender. The PCR fragments were sequenced and assembled. Complete sequence was obtained by using primers 37R and 1118R by primer walking.

The analysis of this "race" PCR and sequencing reactions resulted in the assembly of a contiguous fragment of 1478 base pairs. This sequence contains an open reading frame of 358 amino acids. The full length coding sequence of HG03 was amplified by using primers FL-243F and FL-1429R and cloned into the plasmid vector pCR2.1 by TA cloning (Invitrogen, Carlsbad, CA). It is well within the skilled artisan to determine an appropriate vector for a particular gene transfer or other use.

EXAMPLE 2

Tissue distribution of HG03 RNA transcripts

An approximately 1.8 kb fragment containing HG03 was randomly labelled with ^{32}P-dCTP using the Megaprime DNA Labeling System (Amersham) and used to probe a Human MTN Blot (Clontech Cat # 7760-1, Clontech, Palo Alto, CA, USA). The MTN blot was hybridized in Expresshyb (Clontech) containing 2×10^6 cpm/ml HG03 probe at 65°C overnight and washed to a final stringency of 0.1 X SSC/0.5% SDS at 65°C, and then exposed to X-ray film by autoradiography. The results are shown in Figure 4.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

WHAT IS CLAIMED:

1. A recombinant DNA molecule encoding a polypeptide having an amino acid sequence of SEQ.ID.NO.:2.

2. The DNA molecule of Claim 1 comprising a nucleotide sequence selected from the group consisting of SEQ.ID.NO.:1 and positions 346-1419 of SEQ.ID.NO.:1.

3. A DNA molecule that hybridizes under stringent conditions to the DNA of Claim 1.

4. An expression vector comprising the DNA of Claim 1.

5. A recombinant host cell comprising the DNA of Claim 1.

6. An HG03 protein, substantially free from other proteins, having an amino acid sequence of SEQ.ID.NO.:2.

7. The HG03 protein of Claim 6 containing a single amino acid substitution.

8. The HG03 protein of Claim 7 where the substitution is a conservative substitution.

9. The HG03 protein of Claim 6 containing two or more amino acid substitutions and substantially the same biological activity as HG03.

10. A method for determining whether a substance is capable of binding to HG03 comprising:

(a) providing test cells by transfecting cells with an expression vector that directs the expression of HG03 in the cells;

(b) exposing the test cells to the substance;

(c) measuring the amount of binding of the substance to HG03;

(d) comparing the amount of binding of the substance to HG03 in the test cells with the amount of binding of the substance to control cells that have not been transfected with HG03;

where HG03 has an amino acid sequence of SEQ.ID.NO.:2;

where if the amount of binding of the substance to HG03 in the test cells is greater than the amount of binding of the substance to the control cells, then the substance is capable of binding to HG03.

11. A method for determining whether a substance is capable of binding to HG03 comprising:

(a) providing test cells by transfecting cells with an expression vector that directs the expression of HG03 in the cells;

(b) preparing membranes containing HG03 from the test cells and exposing the membranes from the test cells to the substance;

(c) measuring the amount of binding of the substance to the HG03 in the membranes from the test cells;

(d) comparing the amount of binding of the substance to HG03 in the membranes from the test cells with the amount of binding of the substance to membranes from control cells that have not been transfected with HG03;

where HG03 has an amino acid sequence of SEQ.ID.NO.:2;

where if the amount of binding of the substance to HG03 in the membranes from the test cells is greater than the amount of binding of the substance to the membranes from the control cells, then the substance is capable of binding to HG03.

12. A method for determining whether a substance is capable of binding to HG03 comprising:

(a) providing test cells by transfecting cells with an expression vector that directs ~~the expression of HG03 in the cells~~;

(b) exposing the test cells to a ligand of HG03 under conditions such that the ligand binds to the HG03 in the test cells;

(c) subsequently or concurrently to step (b), exposing the cells to a substance that is suspected of being capable of binding to HG03;

(d) measuring the amount of binding of the ligand to HG03 in the presence and the absence of the substance;

(e) comparing the amount of binding of the ligand to HG03 in the presence and the absence of the substance where a decrease in the amount of binding of the ligand to HG03 in the presence of the substance indicates that the substance is capable of binding to HG03;

where HG03 has an amino acid sequence of SEQ.ID.NO.:2.

13. A method for determining whether a substance is capable of binding to HG03 comprising:

(a) providing test cells by transfecting cells with an expression vector that directs the expression of HG03 in the cells;

(b) preparing membranes containing HG03 from the test cells and exposing the membranes to a ligand of HG03 under conditions such that the ligand binds to the HG03 in the membranes;

(c) subsequently or concurrently to step (b), exposing the membranes from the test cells to a substance;

(d) measuring the amount of binding of the ligand to the HG03 in the membranes in the presence and the absence of the substance;

(e) comparing the amount of binding of the ligand to HG03 in the membranes in the presence and the absence of the substance where a decrease in the amount of binding of the ligand to HG03 in the membranes in the presence of the substance indicates that the substance is capable of binding to HG03;

where HG03 has an amino acid sequence of SEQ.ID.NO.:2.

14. A method for determining whether a substance is a potential agonist or antagonist of HG03 that comprises:

(a) transfecting cells with an expression vector containing HG03;

(b) allowing the transfected cells to grow for a time sufficient to allow HG03 to be expressed;

(c) harvesting the transfected cells and resuspending the cells in assay buffer containing a known labeled agonist of HG03 in the presence and in the absence of the substance;

(d) measuring the binding of the known labeled agonist to HG03;

where if the amount of binding of the known labeled agonist is less in the presence of the substance than in the absence of the substance, then the substance is a potential agonist or antagonist of HG03;

where HG03 has an amino acid sequence of SEQ.ID.NO.:2.

15. A method of identifying agonists of HG03 comprising:

(a) providing cells that expresses both HG03 and a promiscuous G-protein;

(b) exposing the cells to a substance that is a suspected agonist of HG03;

(c) measuring the level of inositol phosphates in the cells;

where an increase in the level of inositol phosphates in the cells in the presence of the substance as compared to the level of inositol phosphates in the cells in the absence of the substance indicates that the substance is an agonist of HG03;

where HG03 has an amino acid sequence of SEQ.ID.NO.:2.

16. A method identifying antagonists of HG03 comprising:

(a) providing cells that expresses both HG03 and a promiscuous G-protein;

(b) exposing the cells to an agonist of HG03;

(c) subsequently or concurrently to step (b), exposing the cells to a substance that is a suspected antagonist of HG03;

(d) measuring the level of inositol phosphates in the cells;

where a decrease in the level of inositol phosphates in the cells in the presence of the substance as compared to the level of inositol phosphates in the cells in the ~~absence of the substance indi~~cates that the substance is an antagonist of HG03;

where HG03 has an amino acid sequence of SEQ.ID.NO.:2.

17. A method of identifying antagonists of HG03 comprising:

(a) providing cells that expresses a chimeric HG03 protein fused at its C-terminus to a promiscuous G-protein;

(b) exposing the cells to an agonist of HG03;

(c) subsequently or concurrently to step (b), exposing the cells to a substance that is a suspected antagonist of HG03;

(d) measuring the level of inositol phosphates in the cells;

where a decrease in the level of inositol phosphates in the cells in the presence of the substance as compared to the level of inositol phosphates in the cells in the absence of the substance indicates that the substance is an antagonist of HG03;

where the HG03 protein has an amino acid sequence of SEQ.ID.NO.:2.

18. An antibody that binds specifically to HG03 where HG03 has an amino acid sequence of SEQ.ID.NO.:2.

19. A method of expressing a truncated version of HG03 protein comprising:

(a) transfecting a host cell with a expression vector that encodes an HG03 protein that has been truncated at the amino terminus;

(b) culturing the transfected cells of step (a) under conditions such that the truncated HG03 protein is expressed.

20. The method of Claim 19 where the truncated HG03 protein is a chimeric HG03 protein.

cDNA POLYNUCLEOTIDE SEQUENCE OF HG03

```
   1 GCAAGTTGTT CCAGTAGTCG CCTGGCAGGA GAATTTGAAA GGGTGCCCCA
  51 AAGGACAATC TCTAAAGGGG TAAGGGAGAT ACCTACCTTG TCTGGTAGGG
 101 GAGATGTTTC GTTTTCATGC TTTACCAGAA AATCCACTTC CCTGCCGACC
 151 TTAGTTTCAA AGCTTATTCT TAATTAGAGA CAAGAAACCT GTTTCAACTT
 201 GAAGACACCG TATGAGGTGA ATGGACAGCC AGCCACCACA ATGAAAGAAA
 251 TCAAACCAGG AATAACCTAT GCTGAACCCA CGCCTCAATC GTCCCCAAGT
 301 GTTTCCTGAC ACGCATCTTT GCTTACAGTG CATCACAACT GAAGAATGGG
 351 GTTCAACTTG ACGCTTGCAA AATTACCAAA TAACGAGCTG CACGGCCAAG
 401 AGAGTCACAA TTCAGGCAAC AGGAGCGACG GGCCAGGAAA GAACACCACC
 451 CTTCACAATG AATTTGACAC AATTGTCTTG CCGGTGCTTT ATCTCATTAT
 501 ATTTGTGGCA AGCATCTTGC TGAATGGTTT AGCAGTGTGG ATCTTCTTCC
 551 ACATTAGGAA TAAAACCAGC TTCATATTCT ATCTCAAAAA CATAGTGGTT
 601 GCAGACCTCA TAATGACGCT GACATTTCCA TTTCGAATAG TCCATGATGC
 651 AGGATTTGGA CCTTGGTACT TCAAGTTTAT TCTCTGCAGA TACACTTCAG
 701 TTTTGTTTTA TGCAAACATG TATACTTCCA TCGTGTTCCT TGGGCTGATA
 751 AGCATTGATC GCTATCTGAA GGTGGTCAAG CCATTTGGGG ACTCTCGGAT
 801 GTACAGCATA ACCTTCACGA AGGTTTTATC TGTTTGTGTT TGGGTGATCA
 851 TGGCTGTTTT GTCTTTGCCA AACATCATCC TGACAAATGG TCAGCCAACA
 901 GAGGACAATA TCCATGACTG CTCAAAACTT AAAAGTCCTT TGGGGGTCAA
 951 ATGGCATACG GCAGTCACCT ATGTGAACAG CTGCTTGTTT GTGGCCGTGC
1001 TGGTGATTCT GATCGGATGT TACATAGCCA TATCCAGGTA CATCCACAAA
1051 TCCAGCAGGC AATTCATAAG TCAGTCAAGC CGAAAGCGAA AACATAACCA
1101 GAGCATCAGG GTTGTTGTGG CTGTGTTTTT TACCTGCTTT CTACCATATC
1151 ACTTGTGCAG AATTCCTTTT ACTTTTAGTC ACTTAGACAG GCTTTTAGAT
1201 GAATCTGCAC AAAAAATCCT ATATTACTGC AAAGAAATTA CACTTTTCTT
1251 GTCTGCGTGT AATGTTTGCC TGGATCCAAT AATTTACTTT TTCATGTGTA
1301 GGTCATTTTC AAGAAGGCTG TTCAAAAAAT CAAATATCAG AACCAGGAGT
1351 GAAAGCATCA GATCACTGCA AAGTGTGAGA AGATCGGAAG TTCGCATATA
1401 TTATGATTAC ACTGATGTGT AGGCCTTTTA TTGTGTGTTG GAATCGATAT
1451 GTACAAAGTG TAAATAAATG TTTCTTTT
```

FIG. 1

PREDICTED POLYPEPTIDE SEQUENCE OF HG03

1 MGFNLTLAKL PNNELHGQES HNSGNRSDGP GKNTTLHNEF DTIVLPVLYL

51 IIFVASILLN GLKVWIFFHI RNKTSFIFYL KNIVVADLIM TLTFPFRIVH

101 DAGFGPWYFK FILCRYTSVL FYNMYTSIV FLGLISIDRY LKVVKPFGDS

151 RMYSITFTKV LSVCVWVIMA VLSLPNIILT NGQPTEDNIH DCSKLKSPLG

201 VKWHTAVTYV NSCLFVAVLV ILIGCYIAIS RYIHKSSRQF ISQSSRKRKH

251 NQSIRVVVAV FFTCFLPYHL CRIPFTFSHL DRLLDESAQK ILYYCKEITL

301 FLSACNVCLD PIIYFFMCRS FSRRLFKKSN IRTRSESIRS LQSVRRSEVR

351 IYYDYTDV*

FIG.2

TRANSLATION OF THE HG03 OPEN READING FRAME

```
        10                  30                  50
GCAAGTTGTTCCAGTAGTCGCCTGGCAGGAGAATTTGAAAGGGTGCCCCAAAGGACAATC

        70                  90                  110
TCTAAAGGGGTAAGGGAGATACCTACCTTGTCTGGTAGGGGAGATGTTTCGTTTTCATGC

        130                 150                 170
TTTACCAGAAAATCCACTTCCCTGCCGACCTTAGTTTCAAAGCTTATTCTTAATTAGAGA

        190                 210                 230
CAAGAAACCTGTTTCAACTTGAAGACACCGTATGAGGTGAATGGACAGCCAGCCACCACA

        250                 270                 290
ATGAAAGAAATCAAACCAGGAATAACCTATGCTGAACCCACGCCTCAATCGTCCCCAAGT

        310                 330                 350
GTTTCCTGACACGCATCTTTGCTTACAGTGCATCACAACTGAAGAATGGGGTTCAACTTG
                                                MetGlyPheAsnLeu

        370                 390                 410
ACGCTTGCAAAATTACCAAATAACGAGCTGCACGGCCAAGAGAGTCACAATTCAGGCAAC
ThrLeuAlaLysLeuProAsnAsnGluLeuHisGlyGlnGluSerHisAsnSerGlyAsn

        430                 450                 470
AGGAGCGACGGGCCAGGAAAGAACACCACCCTTCACAATGAATTTGACACAATTGTCTTG
ArgSerAspGlyProGlyLysAsnThrThrLeuHisAsnGluPheAspThrIleValLeu

        490                 510                 530
CCGGTGCTTTATCTCATTATATTTGTGGCAAGCATCTTGCTGAATGGTTTAGCAGTGTGG
ProValLeuTyrLeuIlePheValAlaSerIleLeuLeuAsnGlyLeuAlaValTrp

        550                 570                 590
ATCTTCTTCCACATTAGGAATAAAACCAGCTTCATATTCTATCTCAAAAACATAGTGGTT
IlePhePheHisIleArgAsnLysThrSerPheIlePheTyrLeuLysAsnIleValVal

        610                 630                 650
GCAGACCTCATAATGACGCTGACATTTCCATTTCGAATAGTCCATGATGCAGGATTTGGA
AlaAspLeuIleMetThrLeuThrPheProPheArgIleValHisAspAlaGlyPheGly
```

FIG. 3A

TRANSLATION OF THE HG03 OPEN READING FRAME

```
      670                 690                 710
CCTTGGTACTTCAAGTTTATTCTCTGCAGATACACTTCAGTTTTGTTTTATGCAAACATG
ProTrpTyrPheLysPheIleLeuCysArgTyrThrSerValLeuPheTyrAlaAsnMet

      730                 750                 770
TATACTTCCATCGTGTTCCTTGGGCTGATAAGCATTGATCGCTATCTGAAGGTGGTCAAG
TyrThrSerIleValPheLeuGlyLeuIleSerIleAspArgTyrLeuLysValValLys

      790                 810                 830
CCATTTGGGGACTCTCGGATGTACAGCATAACCTTCACGAAGGTTTTATCTGTTTGTGTT
ProPheGlyAspSerArgMetTyrSerIleThrPheThrLysValLeuSerValCysVal

      850                 870                 890
TGGGTGATCATGGCTGTTTTGTCTTTGCCAAACATCATCCTGACAAATGGTCAGCCAACA
TrpValIleMetAlaValLeuSerLeuProAsnIleIleLeuThrAsnGlyGlnProThr

      910                 930                 950
GAGGACAATATCCATGACTGCTCAAAACTTAAAAGTCCTTTGGGGGTCAAATGGCATACG
GluAspAsnIleHisAspCysSerLysLeuLysSerProLeuGlyValLysTrpHisThr

      970                 990                1010
GCAGTCACCTATGTGAACAGCTGCTTGTTTGTGGCCGTGCTGGTGATTCTGATCGGATGT
AlaValThrTyrValAsnSerCysLeuPheValAlaValLeuValIleLeuIleGlyCys

     1030                1050                1070
TACATAGCCATATCCAGGTACATCCACAAATCCAGCAGGCAATTCATAAGTCAGTCAAGC
TyrIleAlaIleSerArgTyrIleHisLysSerSerArgGlnPheIleSerGlnSerSer

     1090                1110                1130
CGAAAGCGAAAACATAACCAGAGCATCAGGGTTGTTGTGGCTGTGTTTTTTACCTGCTTT
ArgLysArgLysHisAsnGlnSerIleArgValValValAlaValPhePheThrCysPhe

     1150                1170                1190
CTACCATATCACTTGTGCAGAATTCCTTTTACTTTTAGTCACTTAGACAGGCTTTTAGAT
LeuProTyrHisLeuCysArgIleProPheThrPheSerHisLeuAspArgLeuLeuAsp

     1210                1230                1250
GAATCTGCACAAAAAATCCTATATTACTGCAAAGAAATTACACTTTTCTTGTCTGCGTGT
GluSerAlaGlnLysIleLeuTyrTyrCysLysGluIleThrLeuPheLeuSerAlaCys
```

FIG. 3B

TRANSLATION OF THE HG03 OPEN READING FRAME

```
      1270                 1290                 1310
AATGTTTGCCTGGATCCAATAATTTACTTTTTCATGTGTAGGTCATTTTCAAGAAGGCTG
AsnValCysLeuAspProIleIleTyrPhePheMetCysArgSerPheSerArgArgLeu

      1330                 1350                 1370
TTCAAAAAAATCAAATATCAGAACCAGGAGTGAAAGCATCAGATCACTGCAAAGTGTGAGA
PheLysLysSerAsnIleArgThrArgSerGluSerIleArgSerLeuGlnSerValArg

      1390                 1410                 1430
AGATCGGAAGTTCGCATATATTATGATTACACTGATGTGTAGGCCTTTTATTGTGTGTTG
ArgSerGluValArgIleTyrTyrAspTyrThrAspValEnd

      1450                 1470
GAATCGATATGTACAAAGTGTAAATAAATGTTTCTTTT
```

FIG. 3C

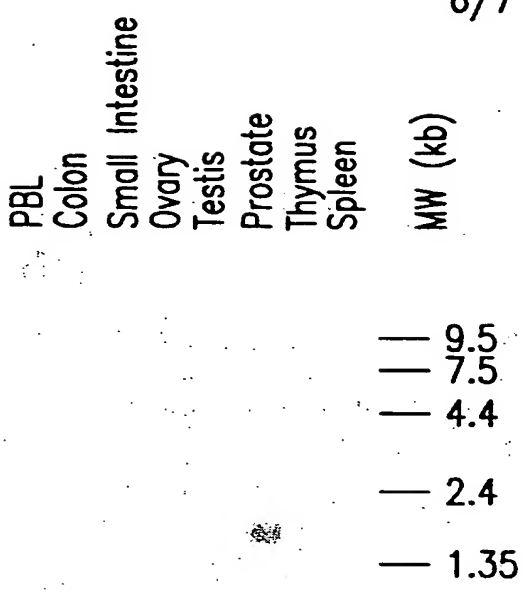

FIG.4A

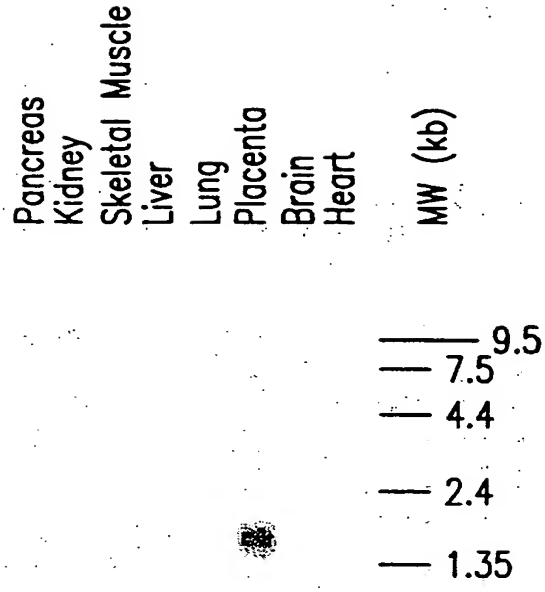

FIG.4B

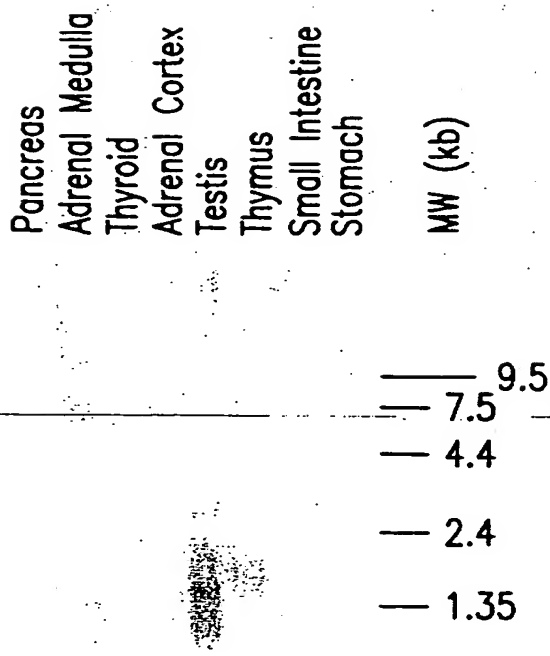

FIG.4C

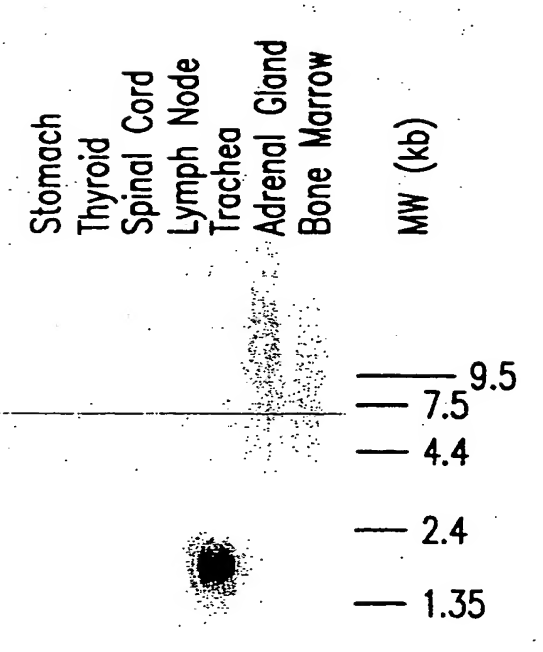

FIG.4D

ALIGNMENT OF THE POLYPEPTIDE SEQUENCES OF HG03 WITH THAT OF THE HUMAN PLATET ACTIVATING FACTOR RECEPTOR.

```
                                                    10        20        30
hPAFR.pep                                   MEPHDSSHMDSEFRYTLFPIVYSIIFVLGVIAN
                                              |  :: : :||   ::|::| |||| ::: |
HG03        MGFNLTLAKLPNNELHGQESHNSGNRSDGPGKNTTLHNEFDTIVLPVLYLIIFVASILLN
                  10        20        30        40        50        60

                 40        50        60        70        80        90
hPAFR.pep   GYVLWVFARLYPCKKFNEIKIFMVNLTMADMLFLITLPLWIVYYQNQGNWILPKFLCNVA
            | ::|:| ::     :|   ::: |:::||::: :|:|: ||:  : | | :  :|| :
HG03        GLAVWIFFHIRNKTSFI——FYLKNIVVADLIMTLTFPFRIVHDAGFGPWYFKFILCRYT
                   70        80        90       100       110

                100       110       120       130       140       150
hPAFR.pep   GCLFFINTYCSVAFLGVITYNRFQAVTRPIKTAQANTRKRGISLSLVIWVAIVGAASYFL
            : ||: | | |::|||:|: :|:  |::|:  ::  :       ||: :|| ::  :   :
HG03        SVLFYANMYTSIVFLGLISIDRYLKVVKPFGDSRMYSITFTKVLSVCVWVIMAVLSLPNI
            120       130       140       150       160       170

                160       170       180       190       200       210
hPAFR.pep   ILDSTNTVPDSAGSGNVTRCFEHYEKGSVPVLIIHIFIVFSFFLVFLIILF-CNLVIIRT
            ||  ||  |      |:  | :     :|       ::   :|:: |:||: | ::| |
HG03        IL—TNGQPTE——DNIHDCSKLKSPLGVKWHTAVTYVNSCLFVAVLVILIGCYIAISRY
              180       190       200       210       220       230

                220       230       240       250       260       270
hPAFR.pep   LLMQPVQQQRNAEVKRRALWMVCTVLAVFIICFVPHHVVQLPWTLAELG-FQDSKFHQAI
            :  : |  :: ||:   : :|:|||: ||:|:|: ::|:|:::|  : | : :: :
HG03        IHKSSRQFISQSSRKRKHNQSIRVVVAVFFTCFLPYHLCRIPFTFSHLDRLLDESAQKIL
                  240       250       260       270       280       290

                280       290       300       310       320       330
hPAFR.pep   NDAHQVTLCLLSTNCVLDPVIYCFLTKKFRKHLTEKFYSMRSSRKCSRATTDTVTEVVVP
              :::|| | : |  |||:|| |: ::| ::| :|  ::|:  :  |:
HG03        YYCKEITLFLSACNVCLDPIIYFFMCRSFSRRLFKK-SNIRTRSESIRSLQSVRRSEVRI
                  300       310       320       330       340       350

                340
hPAFR.pep   FNQIPGNSLKN

HG03        YYDYTDV
```

FIG.5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/17388

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12N 5/10, 5/16, 15/12, 15/62, 15/63, 15/64; C07K 14/705

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.1, 69.7, 70.1, 71.1, 71.2, 471, 325, 252.3, 254.11, 320.1; 530/350; 536/23.1, 23.4, 23.5, 24.3, 24.31

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST search, CAS ONLINE, MEDLINE, EMBASE, BIOSIS, CAPLUS
search terms: receptor HG03, DNA, nucleic acid, expression, recombinant production, chimera, hybrid.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,144,007 A (PFAHL) 01 September 1992 (01/09/92), see entire document.	1-9, 19-20
A	US 4,985,352 A (JULIUS et al) 15 January 1991 (15/01/91), see entire document.	1-9, 19-20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"B"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 22 NOVEMBER 1999	Date of mailing of the international search report 03 DEC 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer PREMA MERTZ Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/17388

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-9, 19-20

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/17388

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/69.1, 70.1, 71.1, 71.2, 471, 325, 252.3, 254.11, 320.1; 530/350; 536/23.1, 23.5, 24.3, 24.31

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-9, 19-20, drawn to a DNA molecule encoding a G-protein coupled receptor HG03, an expression vector, host cell, G-protein coupled receptor HG03, and a method of expressing a truncated G-protein coupled receptor HG03.
Group II, claims 10-17, drawn to a method for determining whether a substance is capable of binding to G-protein coupled receptor HG03.
Group III, claim 18, drawn to an antibody that specifically binds G-protein coupled receptor HG03.
The inventions listed as Groups I-III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:
The inventions listed as Groups I-III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:
Pursuant to 37 C.F.R. § 1.475 (d), the ISA/US considers that where multiple products and processes are claimed, the main invention shall consist of the first invention of the category first mentioned in the claims and the first recited invention of each of the other categories related thereto. Accordingly, the main invention (Group I) comprises the first-recited product, a DNA molecule encoding a G-protein coupled receptor HG03, an expression vector, host cell, G-protein coupled receptor HG03, and a method of expressing a truncated G-protein coupled receptor HG03. Further pursuant to 37 C.F.R. § 1.475 (d), the ISA/US considers that any feature which the subsequently recited products and methods share with the main invention does not constitute a special technical feature within the meaning of PCT Rule 13.2 and that each of such products and methods accordingly defines a separate invention.